PREPARATION AND USE OF *ARTEMIA* AS FOOD FOR SHRIMP AND PRAWN LARVAE

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INTRODUCTION

Although *Artemia* has been known to man for centuries, its use as a food for larval organisms apparently began only in the 1930s, when several investigators found that it could be used as an excellent food for newly hatched fish larvae.[1-3] As aquaculture developed in the 1960s and 1970s, the use of *Artemia* also became more widespread, due to its convenience and its nutritional value for larval organisms. The brine shrimp, *Artemia*, played a significant role in the expansion of the commercial production of shrimp and prawn during the last decade. Being an off-the-shelf and easily usable live food, *Artemia* is the main alternative for the natural diet of shrimp larvae. Nonetheless, it appeared in the late 1970s that the nutritional value of *Artemia*, especially for marine organisms, is not constant, but varies both geographically and temporally, causing unreliable outputs in marine larviculture. Through multidisciplinary studies in Japan and by the *International Study* on *Artemia*, both the causes for nutritional variability in *Artemia* and methods to improve poor-quality *Artemia* were identified.

Furthermore, a better knowledge of the biology of *Artemia* was at the origin of the development of methods for cyst disinfection and decapsulation, and of techniques for its high-density rearing in tank systems. All this resulted in optimized and cost-effective applications of this live food in hatchery production. In this chapter, the updated state of the art in *Artemia* technology is presented.

USE OF *ARTEMIA* NAUPLII

SELECTION OF A SUITABLE STRAIN

To date, *Artemia* cysts are commercially available from various production sources in America, Asia, Australia, and Europe.[4] A knowledge of the hatching and naupliar characteristics of a particular batch of cysts can increase greatly the effectiveness of its usage in a hatchery. Aside from strictly business aspects such as price, payment conditions, shipment and insurance costs, etc., the following criteria should be taken into consideration when selecting a specific cyst product: hatching quality, cyst package condition, naupliar size, and naupliar food value.

Hatching Quality

An acceptable cyst product should contain minimal quantities of impurities[5] (e.g., sand, cracked shells, plumes, salt crystals, etc.). Hatching synchrony must be high: when incubated in 35 ppt seawater at 25°C, the first nauplii should appear after 13 to 16 h of incubation and the last nauplii should have hatched within less than 8 h thereafter.[6,7] This is important because the practical aquaculturist normally wishes to harvest the nauplii and feed them to the cultured shrimp as soon as possible. When hatching synchrony is low, first-hatched nauplii will have consumed much of their energy reserves by the time the last nauplii have hatched.[8] Hatching efficiency (the number of nauplii hatched per gram of cysts) and hatching percentage (the total percentage of the cysts that actually hatch) vary substantially among different batches and obviously account for much of the price differences. Hatching may be as low as 100,000, while premium quality cysts from Great Salt Lake yield over 250,000 nauplii per gram of cysts and Brazilian or Vietnamese cyst batches may have an even higher

CRC Handbook of Mariculture. 1993. Vol. I. Crustacean Aquaculture (2nd Edition)
J.P. McVey (Ed.). CRC Press, Inc., Boca Raton, Florida, USA. 526p. p.61-93.

TABLE 1
Size and Individual Dry Weight of *Artemia* Nauplii from Different Cyst Sources Hatched in Standard Conditions (35 ppt, 25°C)

Cyst source	Nauplius	
	Length (mm)	Dry weight (μg)
San Francisco Bay, CA	428	1.63
Macau, Brazil	447	1.74
Great Salt Lake, UT	486	2.42
Shark, Bay, Australia	458	2.47
Chaplin Lake, Canada	475	2.04
Buenos Aires, Argentina	431	1.72
Lavalduc, France	509	3.08
Bohai Bay, PR China	515	3.09
Margherita di Savoia, Italy	517	3.33
Reference *Artemia* cysts	448	1.78

Data compiled from Vanhaecke, P. and Sorgeloos, P., *Aquaculture*, 30, 43, 1983; *Mar. Ecol.*, 3, 303, 1980.

yield, i.e., over 300,000. The possible biological mechanisms responsible for these differences have been reviewed.[9]

Cyst Packaging Conditions

Cysts should be dehydrated before storage (moisture content preferably <5%) and kept away from humid conditions, since a higher moisture content reduces hatchability in time.[9] Storage under vacuum or nitrogen is important, as the presence of oxygen will induce the formation of free radicals which eventually reduce hatchability.[10] Properly packed cyst containers are preferentially kept at low temperature for acclimation. When frozen, the cysts should be held 1 week at room temperature before they are incubated in seawater for hatching.[11]

Naupliar Size

The nutritional effectiveness of a food organism is first determined by its ingestibility and, as a consequence, by its size and form. With the exception of *Penaeus* spp. larvae, which are phytoplankton feeders initially, most decapod larvae can be reared on *Artemia* nauplii for their complete development.[12] As long as prey size does not interfere with the ingestion mechanism of the predator, the use of larger nauplii (with a higher individual energy content) will be beneficial, since the predator will spend less energy in taking up a smaller number of larger nauplii to fulfill its energy requirements. Data on the biometrics of nauplii from various *Artemia* strains are given in Table 1.

Naupliar Food Value

In the late 1970s and 1980s, when many fish and shrimp farmers started to go commercial, unexpected problems were reported when switching from one source of *Artemia* to another. The situation became more critical when very significant differences in production yields were obtained with distinct batches of *Artemia* of the same geographical origin. Multidisciplinary studies in Japan and by the *International Study* on *Artemia* revealed that the concentration of the essential fatty acid eicosapentaenoic acid (EPA) in nauplii determines

the nutritional value of a particular batch for the larvae of various marine fish and shrimp species. Again, levels of this essential fatty acid (EFA) vary tremendously from strain to strain and even from batch to batch, the causal factor being the fluctuations of the quality of the primary producers available to the adult population.[13] Following these observations, appropriate techniques were developed for improving the lipid profile of deficient *Artemia* strains (see further). Commercial provisions of *Artemia* cysts containing high EPA levels are limited and, as a consequence, these cysts are much more expensive than common cyst batches. Therefore, the use of the high-EPA cysts should be restricted to the feeding period when feeding of freshly hatched nauplii of a small size is required.

By contrast to fatty acids, the amino acid composition of *Artemia* nauplii seems to be remarkably similar from strain to strain,[14] suggesting that it is not environmentally determined in the same manner as the fatty acids. The levels of essential amino acids in *Artemia* are generally not a major problem in view of its nutritional value, but sulfur amino acids such as methionine are the first limiting amino acids.[15]

The presence of several proteolytic enzymes in developing *Artemia* embryos and *Artemia* nauplii[12,16,17] and the high proteolytic activity in nauplii[18] has led to speculation that these exogenous enzymes play a significant role in the breakdown of the *Artemia* nauplii in the digestive tract of marine larvae.[12] This has become an important question in view of the relatively low levels of digestive enzymes in many first-feeding larvae and the inferiority of prepared feeds vs. live prey.

The levels of several minerals in *Artemia* as reported in the literature have been summarized recently.[12] The mineral requirements of marine organisms are poorly known, but may be met by the seawater that they ingest. The main concern about the mineral composition of *Artemia* is whether it meets the requirements of freshwater organisms in culture, about which our knowledge is also poor. A recent study of the variability of 18 minerals and trace elements in *Artemia* cysts revealed that the levels of selenium and manganese were the most variable and that selenium, in some cases, may not be present in sufficient quantity.[19]

HATCHING TECHNIQUES

Although the hatching of *Artemia* cysts is basically very simple, several parameters need to be taken into consideration for successful hatching of kilogram quantities of cysts, as is needed on a daily basis in large hatcheries. The best hatching results are achieved with containers with a conical bottom, aerated from the bottom with air lines (Figure 1). Cylindrical or square-bottomed tanks will have dead spots in which *Artemia* cysts and nauplii will accumulate and suffer from depletion of oxygen. The aeration intensity must be sufficient to maintain oxygen levels above 2 mg/l, preferably 5 mg/l. The temperature of the seawater is preferentially kept in the range of 25 to 28°C. The pH must remain around 8 during the hatching process. If needed, the buffer capacity of the water is increased by adding 1 g $NaHCO_3$/l (this is particularly needed when hatching at salinities lower than 30 ppt; the minimal required salinity is 5 ppt). Cyst densities may be as high as 5 g/l for small volumes (<20 l), but should be decreased to a maximum of 2 g/l for large volumes (>100 l). *Artemia* cyst shells may be loaded with germs of bacteria and fungi.[20] These may be removed by disinfection using bleach. A minimum treatment consists of a 30-min soak of the cysts in a 200-ppm hypochlorite solution (fresh or seawater) prior to incubation for hatching.[21] This treatment, however, may not kill germs present in the alveolar and cortical layer of the outer shell. Complete sterilization can be achieved through cyst decapsulation (see further). Strong illumination (about 2000 lx at the water surface) is essential, at least during the first hours after complete hydration, in order to trigger the start of the hatching mechanism.[22]

After hatching and prior to feeding the nauplii to shrimp larvae, the nauplii should be separated from empty cyst shells, unhatched cysts, debris, microorganisms, and hatching

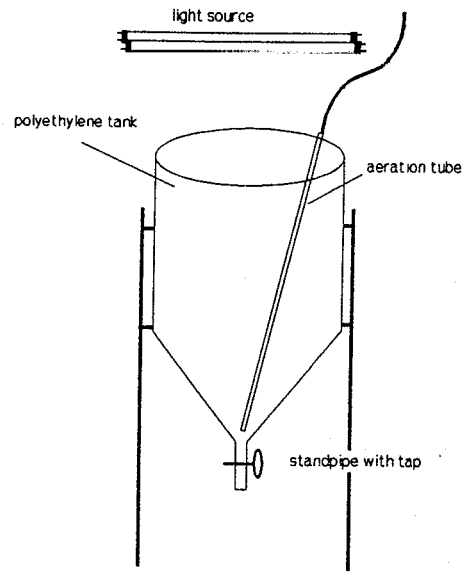

FIGURE 1. Schematic drawing of a typical hatching container.

metabolites. Five to 10 min after the aeration is switched off, cyst shells will float, while nauplii and unhatched cysts will concentrate at the bottom. Since nauplii are positively phototactic, their concentration can be improved by shading the upper part of the hatching tank and focusing a light source on the bottom. First, unhatched cysts and other debris that have accumulated underneath the nauplii are siphoned or drained off. Then the nauplii are collected on a submerged fine-mesh screen (<150 μm) and rinsed thoroughly with tap water in order to remove possible contaminants and hatching metabolites such as glycerol.[23]

DECAPSULATED CYSTS

The hard shell that encysts the dormant *Artemia* embryo can be removed by chemical means in a procedure called decapsulation. Decapsulated cysts offer a number of advantages. First, cyst shells are not introduced into the culture tanks. When hatching undecapsulated cysts, the complete separation of *Artemia* nauplii from their shells is not always possible. Unhatched cysts and empty shells in the larval tanks can cause deleterious effects when they are ingested by the predator. They are not digested and may obstruct the gut.[24] Second, nauplii that hatched out of decapsulated cysts have a higher energy content than regular instar I nauplii because they do not spend energy to break out of the shell.[25] Finally, in some cases where cysts have a very low energy content, the hatchability might be improved again because of the lower energy requirement to break out of a decapsulated cyst.[26]

Decapsulated cysts can also be used as a direct food source for shrimp, offering the following advantages: they are smaller particles than instar I nauplii[27] and are free from bacteria.[28] A major handicap of decapsulated cysts is that they are nonmoving, nonbuoyant particles.[29] Extra aeration is needed to keep those particles in suspension. Older shrimp larvae, however, are mainly bottom feeders and do not encounter this problem.

The decapsulation procedure involves the hydration of the cysts, removal of the cyst envelope in a hypochlorite solution,[30] and washing and deactivation of the hypochlorite.

TABLE 2
Procedure for Decapsulation of *Artemia* Cysts

1. Hydrate cysts by placing them in fresh or salt water, bubbled in a separated funnel for 2 h at 25°C.
2. Collect cysts on 125-μm mesh sieve, rinse, and transfer to hypochlorite solution.
3. The hypochlorite solution, made up in advance of either liquid bleach, NaOCl, or bleaching powder, $Ca(OCl)_2$ should consist of 0.5 g active hypochlorite product (activity normally labeled on the package) per gram of cysts, plus an alkaline product to keep the pH >10 (0.15 g technical grade NaOH for liquid bleach, 0.67 g $NaCO_3$ or 0.4 g CaO for bleaching powder, per gram of cysts) and enough seawater to make the final solution up to 14 ml/g of cysts. Dissolve bleaching powder first before adding alkaline product.
4. Keep the solution at 15 to 20°C by addition of ice to the surrounding water bath, add the hydrated cysts, and stir for 5 to 15 min. Check temperature regularly, since the reaction is exothermic; never exceed 40°C. When cyst color becomes gray (with powder bleach) or orange (with liquid bleach), or when microscopic examination shows the cyst envelope to be completely dissolved, remove the cysts and rinse them with water on a 120-μm screen until the chlorine smell disappears.
5. Totally deactivate the hypochlorite by dipping the cysts (<1 min) in either 0.1 *N* HCl or 0.1% $Na_2S_2O_3$ solution, then rinse again with water.

The resulting decapsulated cysts can then be fed immediately to shrimp larvae, hatched into nauplii, or dehydrated for storage. The detailed procedure is described in Table 2.

COLD STORAGE

For a long time, farmers have overlooked the fact that an *Artemia* nauplius is not feeding in its first stage of development and thus consumes its own energy reserves.[8] At the high water temperatures which are applied for cyst incubation, the freshly hatched *Artemia* nauplii develop into the second larval stage within a matter of hours. It is important to feed first-instar nauplii to the predator rather than starved second-instar metanauplii, which have consumed 25 to 30% of their energy reserves within hours. Instar II *Artemia* are less visible, as they are transparent, and they are larger and swim faster than first-instar larvae,[31] as a result of which they are less acceptable as prey. Furthermore, they contain lower amounts of free amino acids, so they are less digestible and their lower individual organic dry weight and energy content will reduce the energy uptake by the predator per hunting effort.[32] All this will be reflected in reduced growth of the larvae and an increased *Artemia* cyst bill (as about 20 to 30% more cysts need to be hatched to feed the same weight of starved metanauplii to the predator). Molting of the *Artemia* nauplii to the second-instar stage may be avoided and their energy metabolism greatly reduced (Figure 2) by storage of the freshly hatched nauplii at a temperature below 10°C in densities of up to 8 million nauplii per liter, for periods up to 24 h.[32] A slight aeration is needed in order to prevent the nauplii from accumulating at the bottom of the tank, where they would suffocate. By applying 24-h cold storage (using styrofoam-insulated tanks and blue ice packs for cooling), commercial hatcheries are able to economize their *Artemia* cyst-hatching efforts[12] (e.g., fewer tanks, bigger volumes, maximum one hatching and harvest per day). Furthermore, cold storage allows the farmer to ensure the availability of a better quality product and, at the same time, to consider more frequent food distributions. This appears to be beneficial for fish and shrimp larvae, as food retention times in the larviculture tanks can be reduced and hence growth of the *Artemia* in the culture tank can be avoided. With poor hunters such as the larvae of the tiger shrimp *Penaeus monodon*, feeding cold-stored *Artemia* results in much more efficient food uptake.

ENRICHMENT TECHNIQUES

As mentioned above, the main factor affecting the nutritional value of *Artemia* as a food source for marine larval organisms is the content of the EFA 20:5n-3 (EPA). Moreover, the

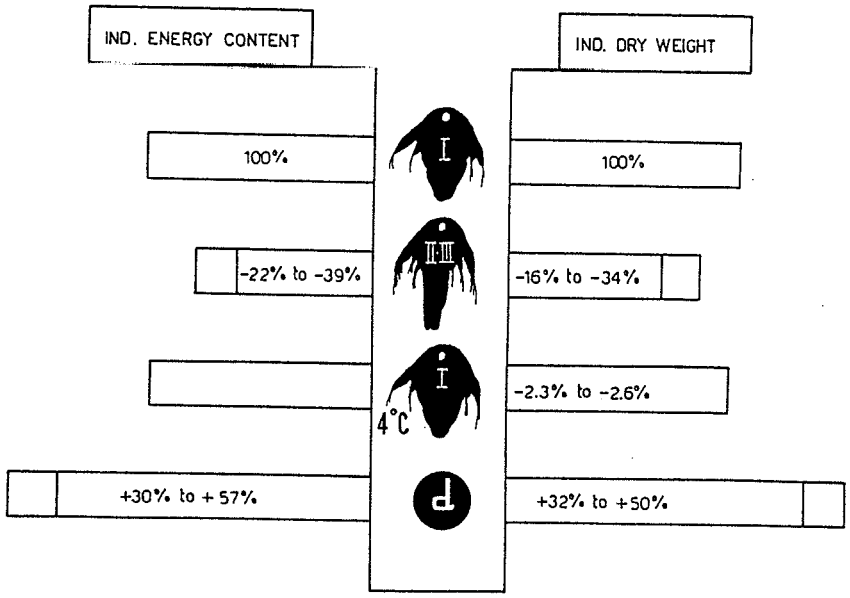

FIGURE 2. Change in energy content and dry weight of different forms of *Artemia* (newly hatched Instar I nauplii are considered to have 100% values for these variables). The percent decrease or increase from 100% is shown for (top to bottom) Instar I, Instar II–III metanauplii, 24-h cold-stored Instar I nauplii, and decapsulated cysts. (From Léger, Ph., Bengtson, D. A., Sorgeloos, P., Simpson, K. L., and Beck, A. D., in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 357. With permission.)

content of the other EFA, docosahexaenoic acid (22:6n-3 or DHA), is always very low in *Artemia*. In view of the fatty acid deficiency in *Artemia*, research has been conducted to modify its lipid composition. Because of its primitive characteristics, *Artemia* facilitates a very convenient way to manipulate its biochemical composition. Since *Artemia* is nonselective in taking up particulate matter once it has molted into the second larval stage, i.e., about 8 h following hatching, simple methods could be developed to incorporate particulate products into *Artemia* prior to offering it as prey to the predator larva. This method of bioencapsulation is called *Artemia* enrichment or boosting. British, Japanese, and Belgian researchers developed enrichment products and procedures using selected microalgae and/or microencapsulated products, yeast, and/or emulsified preparations, as well as self-emulsifying concentrates and/or microparticulate products (for a review, see Reference 12). The highest enrichment levels are obtained when using emulsified concentrates (Figure 3): freshly hatched nauplii are transferred to the enrichment tank at a density of 100 (for enrichment periods of >24 h) or 300 nauplii per milliliter (maximum 24-h enrichment period). The enrichment medium consists of disinfected seawater maintained at 25°C. The enrichment emulsion is added in consecutive doses of 300 ppm every 12 h. A strong aeration, using airstones, is of major importance to maintain dissolved oxygen levels above 4 ppm. Enriched nauplii are harvested after 24 to 48 h, thoroughly rinsed, and stored at temperatures below 10°C in order to ensure that highly unsaturated fatty acids (HUFAs) are not metabolized during storage[33] (see Figure 4). In this way, maximal incorporation levels of EPA and DHA can be reached, well above the maximal concentrations found in natural strains. Through

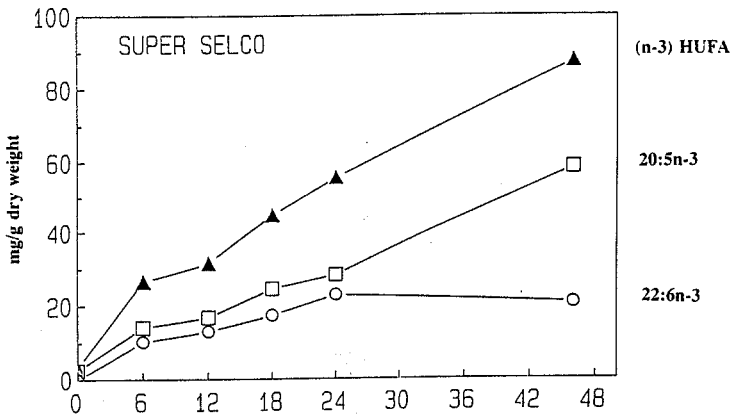

FIGURE 3. HUFA levels in Great Salt Lake (UT) *Artemia* metanauplii enriched with the self-emulsifying concentrate, SUPER SELCO (*Artemia* Systems, N.V., Ghent, Belgium).

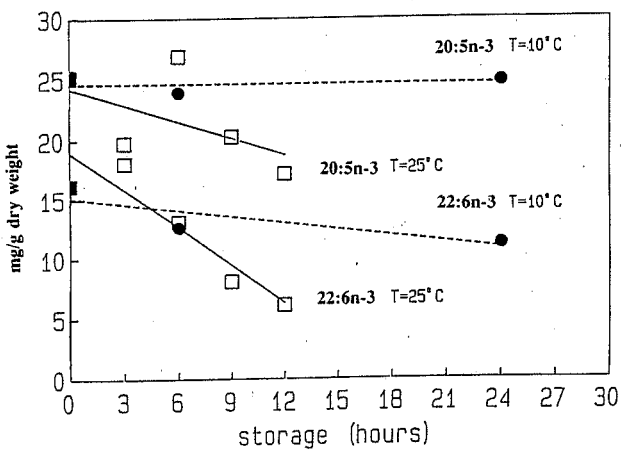

FIGURE 4. HUFA levels in SUPER SELCO-enriched *Artemia* metanauplii during storage at 10 and 25°C.

this technique, other components such as vitamins, phospholipids, pigments, and therapeutics can be bioencapsulated in *Artemia*.[29]

USE AND CULTURE OF ONGROWN *ARTEMIA*

Examples of applications of ongrown *Artemia* as food for fish and crustacean larvae are well documented in the literature.[12] Until recently, however, these applications were never taken up at an industrial level because of the limited availability of live or frozen biomass, its high cost, and variable quality.[34] The possibilities for setting up intensive (man-managed pond) and superintensive (tank) production systems of brine shrimp in or nearby the aquaculture farm have resulted in increased interest in *Artemia* biomass during the last decade.

Although tank-produced *Artemia* biomass is far more expensive than pond-produced, its advantages for application are manifold:

1. Ongrown *Artemia* are available year round, independent of climate or season.
2. Specific stages (juveniles, preadults, and adults) can be harvested as a function of possible size preferences of the predator.
3. The quality of the *Artemia* can be better controlled (e.g., nutritionally, free from diseases).

Besides the advantages of superintensive-produced biomass, superintensive culture techniques offer two main advantages compared to pond production techniques. First, there is no restriction with regard to production site or time (the culture procedure does not require high-saline waters or specific climatological conditions). Second, the controlled production can be performed with very high densities of brine shrimp, e.g., several thousand animals per liter vs. a maximum of a few hundred animals per liter in outdoor culture ponds. As a consequence, very high production yields per volume of culture medium can be obtained with tank-rearing systems.

Before describing the various culture techniques, we review briefly the abiotic and biotic conditions relevant for *Artemia* culture. More detailed information is given in the *Artemia* manual[21] and in Lavens and Sorgeloos.[35]

ABIOTIC AND BIOTIC CULTURE CONDITIONS

Ionic Composition of the Culture Media

Besides natural seawater or brine, several artificial media with different ionic compositions are used frequently in indoor installations for brine shrimp production. Examples of the composition of such media are given in Table 3.

Temperature, Salinity, pH, and Oxygen Concentration

For most strains of *Artemia*, a common range of preference for temperature is 19 to 25°C and for salinity, 35 to 110 ppt[36] (see also Table 4). In the literature, it is generally accepted that the pH tolerance for *Artemia* ranges from 6.5 to 8.[37,38]

With regard to oxygen, only very low concentrations of less than 2 ppm will limit the production of biomass.[39] For optimal production, O_2 concentrations higher than 2.5 ppm are suggested.[40] Levels of O_2 higher than 5 ppm, on the other hand, will result in the production of pale animals, possibly with a lower individual dry weight, which may therefore be less perceptible and attractive for the predators.

Water Quality

The quality of the culture medium will be affected by particles as well as soluble substances. Although no exact data on maximal concentrations are available, it is generally accepted that high levels of suspended solids will affect production characteristics, either by interfering with food uptake and propulsion by *Artemia* or by bacterial growth and resulting competition for oxygen. Soluble waste products give rise to toxic nitrogen compounds (e.g., NH_3-N, NO_2-N, and NO_3^--N). Concentrations up to 1000 ppm NH_4^+ and 320 ppm NO_2^- did not affect the survival (LC_{50}) or growth of Great Salt Lake (GSL) *Artemia*.[41,42] Hence, it is very unlikely that N components will interfere directly with the *Artemia* cultures. Nevertheless, the presence of soluble substances should be restricted as much as possible because they are an ideal substrate for bacteria.

TABLE 3
Artificial Seawaters Used for Tank Production of *Artemia*

Solution A					
Instant Ocean		**Dietrich and Kalle[a]**		**ARC**	
Cl^-	18.4	NaCl	23.9	NaCl	31.08
Na^-	10.22	$MgCl_2 \cdot 6\ H_2O$	10.83	$MgCl_2$	6.09
SO^{2-}	2.516	$CaCl_2$ anhydric	1.15	$CaCl_2$	1.53
Mg^{2-}	1.238	$SrCl_2 \cdot 6\ H_2O$	0.004	KCl	0.97
K^-	0.39	KCl	0.682	$MgSO_4$	7.74
Ca^{2-}	0.37	KBr	0.099	$NaHCO_3$	1.80
HCO_3^-	0.142				
H_2O	1000	H_2O distilled	856	H_2O	1000

Solution B	
$NaSO_4 \cdot 10\ H_2O$	9.06
$NaHCO_3$	0.02
NaF	0.0003
H_3BO_3	0.0027
H_2O distilled	1000

Note: All data in grams; H_2O in ml; ARC, Artemia Reference Center.

[a] Solutions A and B are prepared separately, then mixed, and strongly aerated for 24 h.

Data compiled from Lavens, P., Ph.D. thesis, State University of Ghent, Belgium, 1989.

Feeding Conditions

Artemia is a continuous, nonselective, particle-filtrating organism. Various factors may influence the feeding behavior of *Artemia* by affecting the filtration rate, ingestion rate, and/or assimilation: the quality and quantity of the food offered, the developmental stage of the larvae, and the culture conditions. More detailed information about these processes is given in Lavens[41] and Coutteau and Sorgeloos.[43]

Selection of Suitable Diets

Artemia can take up and digest exogenous microflora as part of its diet. Bacteria and protozoans which develop easily in the *Artemia* cultures are able to biosynthesize essential nutrients, as they use the supplied brine shrimp food as a substrate; in this way, they compensate for possible deficiencies in the composition of the diet.[44]

Interference by bacteria makes it difficult to identify nutritionally adequate diets as such, since growth tests are difficult to run under axenic conditions. As a consequence, nutritional composition of the diet does not play the most critical role in the selection of foods suitable for the high-density culture of brine shrimp. More important criteria are

- Availability and cost
- Particle-size composition (<50 μm)
- Digestibility

TABLE 4
The Effect of Temperature on Different Production Parameters for Various Geographical Strains of *Artemia*

Geographical strain	Temperature (°C)					
	20	22.5	25	27.5	30	32.5
San Francisco Bay, CA						
Survival (%)	97	97	94	91	66	—[i]
Biomass production (%)[a]	75[a]	101	100	94	88	—
Specific growth rate[b]	0.431	0.464[d]	0.463[d,e]	0.456[e]	0.448[f]	—
Food conversion[c]	3.89	3.35[d]	3.64[e]	3.87[e]	4.15[f]	—
Great Salt Lake, UT						
Survival (%)	77	85	89	89	87	88
Biomass production (%)	69	104	122	128	135	78
Specific growth rate	0.392[b]	0.437[f]	0.454[e]	0.460[d,e]	0.465[d]	0.406[g]
Food conversion	3.79[f]	2.90[e]	2.65[d,e]	2.52[d]	2.40[d]	4.14[g]
Chaplin Lake, Canada						
Survival (%)	72	75	77	65	50	—
Biomass production (%)	78	102	108	106	90	—
Specific growth rate	0.422[f]	0.452[d,e]	0.459[d]	0.456[d]	0.437[e,f]	—
Food conversion	3.42[d]	3.00[d]	3.03[d]	3.11[d]	3.72[d]	—
Manaure, Colombia						
Survival (%)	94	94	91	93	86	77
Biomass production (%)	52	102	112	110	104	105
Specific growth rate	0.379[f]	0.451[e]	0.462[d]	0.460[d,e]	0.454[d,e]	0.455[d,e]
Food conversion	5.82[e]	3.43[d]	3.36[d]	3.40[d]	3.62[f]	3.56[d]
Bueonos Aires, Argentina						
Survival (%)	96	90	94	91	80	—
Biomass production (%)	93	113	115	98	96	—
Specific growth rate	0.462[e]	0.430[d]	0.485[d]	0.468[e]	0.465[e]	—
Food conversion	2.78[d]	2.66[d]	3.29[e]	3.38[e]	—	
Larnaca, Cyprus						
Survival (%)	98	94	89	62	3	—
Biomass production (%)	61	94	95	83	0	—
Specific growth rate	0.384[e]	0.430[d]	0.432[d]	0.417[f]	0[h]	—
Food conversion	4.29[e]	3.23[d]	3.43[d]	3.93[e]	—	—
Shark Bay, Australia						
Survival (%)	97	96	97	92	68	—
Biomass production (%)	48	67	84	75	66	—
Specific growth rate	0.332[f]	0.369[e]	0.393[d]	0.381[d,e]	0.367[e]	—
Food conversion	6.49[f]	5.35[d,e]	4.57[d]	5.08	5.81[e,f]	—
Tientsin, PR-China						
Survival (%)	95	94	91	93	84	54
Biomass production (%)	41	61	80	92	85	16
Specific growth rate	0.299[f]	0.343[e]	0.371[d]	0.387[d]	0.378[d]	0.208[g]
Food conversion	7.22[f]	5.42[e]	4.46[d,e]	3.84[d]	4.22[d,e]	22.04[g]

[a] Expressed as percent recorded for the *Artemia* reference strain (San Francisco Bay, batch 288-2596) at 25°C after 9 d of culturing on a diet of *Dunaliella* cells.

[b] Specific growth rate $k = \ln(W_t - W_o) \cdot T^{-1}$ with T = duration of experiment in days (=9).

[c] Food conversion $= F \cdot (W_t - W_o)^{-1}$ where F = µg dry weight *Dunaliella* offered as food; W_t = µg dry weight *Artemia* biomass after 9-d culturing; W_o = µg dry weight *Artemia* biomass at start of experiment.

[d–h] Means with the same superscript are not significanlty different at the $p < 0.05$ level.

[i] Not analyzed.

Data compiled from Vanhaecke, P. and Sorgeloos, P., *Ann. R. Soc. Zool. Belg.*, 7, 119, 1989.

- Consistency in composition among different batches and storage capacity
- Solubility (minimal)
- Food conversion efficiency (FCE)
- Buoyancy

Commonly used food sources are

Live microalgae[45] — The main restriction in using this feed source is its limited availability. Mass culturing of suitable algae for *Artemia* is not realistic economically, so their use can be considered only in those places where the algal production is an additional feature of the main activity.

Dried algae — In most cases, algal meals give satisfactory growth performance,[46-50] especially when water quality conditions are kept optimal. Drawbacks in the use of these feeds are their high cost (7 $12/kg), and high fraction of water-soluble components, which cannot be ingested by the brine shrimp but interfere with the water quality of the culture medium.

Bacteria and yeasts — Single-cell proteins (SCP) have several characteristics which make them an interesting alternative for microalgae:

1. The cell diameter is mostly smaller than 20 μm.
2. The nutritional composition is fairly complete.[51]
3. The rigid cell walls prevent the leakage of water-soluble nutrients in the culture medium.
4. Products are commercially available at acceptable cost (e.g., commonly used in cattle feeds[52,53]).

The highly variable production yields, which often occur when feeding a yeast monodiet, are assigned to nutritional deficiencies of the yeast diet,[44,54,55] and should therefore be met by supplementation with other diets. For some SCP, digestibility by the *Artemia* can be a problem as well. Complete removal of the complex and thick yeast cell wall by enzymatic treatment and/or supplementation of the diet with live algae significantly improves the assimilation rate and growth rate of the brine shrimp.[56]

Waste products from the food industry — Nonsoluble waste products from agricultural crops or from the food-processing industry (e.g., rice bran, corn bran, soybean pellets, and lactoserum) appear to be a very suitable feed source for high-density culturing of *Artemia*.[57] Their main advantages are low cost and worldwide availability. In most cases, however, these commercially available feeds do not meet the particle size requirements for *Artemia* and an extra treatment is needed (e.g., squeezing through a filter bag or micronization).

Strain Selection

Based on laboratory results (Table 5), guidelines are provided for strain selection as a function of optimal temperature and culture performance. The optimal strain should be selected according to specific culture conditions.

Density of *Artemia*

Depending on the applied culture technique, inoculation densities up to 5000 larvae per liter for batch culture,[58] 10,000 for closed flow-through culture,[59] and 18,000 for open flow-through culture[60] can be maintained without interference with survival and growth (Table 6). Maximum densities cause no real interference with behavior. Of course, each culture has its maximum carrying capacity; above these optimal densities, culture conditions become suboptimal (water quality deterioration, lower individual food availability) and growth and survival decrease.

TABLE 5
Survival and Growth of Different *Artemia* Strains Cultured in Standard Laboratory Conditions with *Dunaliella* (DUN) or Rice Bran (RB) as Food for 7 Days

Geographical strain	Survival at day 7 (%)		Growth (%) recorded for reference strain San Francisco Bay (288-2596)	
	DUN diet	RB diet	DUN diet	RB diet
Larnaca Salt Lake (Cyprus)	70[a]		88[a]	
Santa Pola (Spain)	76[a]		88[a]	
Salin du Giraud (France)	66[a]		90[a]	
China (unknown locality)	84	83	91[a]	
Shark Bay (Australia)	90 (84)[b]	51[a]	95 (96)[b]	89[a]
San Francisco Bay (CA; batch 236-2016)	98		96	
Macau (Brazil, harvested in March 1978)	84 (96)[b]	90	96 (98)[b]	102
Barotac Nuevo (Philippines)	86	86	97	103
Tientsin (China)	96	86	98	
San Francisco Bay (CA; batch 933-235)	94		99	
Aigues Mortes (France)	90		99	
Izmir (Turkey)	96	88	101	108
Margherita di Savoia (Italy)	96	88	102	99
San Felix (Spain)	74[a]		102	
Macau (Brazil, harvested in May 1978)	94		103	
Bonaire (Netherlands Antilles)	66[a]	90	104	104
San Pablo Bay (CA; batch 1628)	90	92	105	106
Port Araya (Venezuela)	90	81	107	112
Eilat (Israel)	92[a]		107	
Lavalduc (France)	70[a]	93	109	96
Adelaide (Australia)	88 (88)[b]		113[a] (113)[b]	
Manaure (Colombia)	90	85	115[c]	129[c]
Bahia Salinas (Puerto Rico)	88	74[a]	122[c]	122[c]
Great Salt Lake (UT; harvested in 1977)	94	86	119[c] (125)[b]	115[c]
Buenos Aires (Argentina)	72[a]	80	126[c]	124[c]
Galerazamba (Columbia)	98	87	126[c]	133[c]
Great Salt Lake (UT; harvested in 1966)	66[a]		127[c]	
Chaplin Lake (Canada)	88	77	130[c]	130[c]

[a] Significantly different from the reference strain at the 0.01 level.
[b] Result of replicate test is in parenthesis.
[c] Significantly different from the reference strain at the 0.05 level.

Data compiled from Vanhaecke, P. and Sorgeloos, P., *Mar. Ecol.*, 3, 303, 1980; Vanhaecke, P. and Sorgeloos, P., *Ann. R. Soc. Zool. Belg.*, 7, 119, 1989.

INTENSIVE *ARTEMIA* CULTURE

Infrastructure

Tank and Aeration Design

It is assumed that *Artemia* can be reared in containers of any possible shape as long as the installed aeration ensures proper oxygenation and adequate mixing of feed and animals through the total culture volume. Aeration should not be too strong. Thus, aeration and tank design cannot be considered separately, as the circulation pattern is determined by the combination of both. A wide variety of culture tanks have proven to be suitable. For small cultures up to 1 m^3, rectangular tanks are most convenient. They can be aerated with an air-water lift (AWL) system or, simpler, by an aeration collar mounted around a central standpipe (Figure 5) or by perforated PVC tubes fixed to the bottom of the tank (Figure 6).

TABLE 6
Production Characteristics of Great Salt Lake *Artemia* Cultures as a Function of Different Animal Densities at Start of Culture

Density (individuals/ml)	Survival (%)			Growth (mm)			Biomass (g wet weight/l)	FCE (g dry food/g wet weight biomass)
	Day 7	Day 10	Day 14	Day 7	Day 10	Day 14	Day 14	Day 14
5	82	77	58	3.3	4.7	6.1	15.5	0.66
8	66	50	56	3.2	4.8	5.2	14.5	0.75
10	95	73	72	1.8	2.9	3.5	11.6	0.67
13	61	32	28	—	—	—	8.0	1.13
15	55	41	34	2.0	2.9	3.8	10.0	1.17
18	58	37	29	—	—	—	5.8	1.34
40	74	12	—	1.5	2.3	—	—	—

Data compiled from Lavens, P., Ph.D. thesis, State University of Ghent, Belgium, 1989.

For large volumes (>1 m^3), it is advantageous to switch to cement tanks covered on the inside with impermeable plastic sheets or coated with special paint. These large tanks are traditionally operated as raceway-type systems. They are oblong, with widths confined to ± 1.5 m and a height-to-width ratio kept close to 1:2 (Figure 7). The length is then chosen according to the desired volume. The corners of the tank may be curved to prevent dead zones where sedimentation can occur. A partitioning to which AWLs are fixed is installed in the middle of the tank and ensures a combined horizontal and vertical movement of the water, which results in a screw-like flow pattern. If axial blowers are used for aeration, the water depth should not exceed 1.2 m, to ensure optimal water circulation.

Heating

When the ambient temperature is below optimal culture values, heating is imperative. Small volumes (<1 m^3) are most conveniently heated using electric thermoregulated resistances. Depending on the ambient temperature, a capacity of up to 1000 W/m^3 must be provided. For larger volumes, a heat exchanger consisting of a thermostat-controlled boiler with copper tubing under the bottom of the culture tank is recommended. Heat losses might be avoided by insulating the tank with styrofoam and covering the surface with plastic sheets.

Feeding Strategy and Feed Distribution Apparatus

The feed concentration of inert feed in a culture tank is commonly determined by measuring the transparency of the water with a simplified secchi disc.[57,61] Experience has shown that optimal feed levels are at 15 to 20 cm transparency during the first culture week and 20 to 25 cm the following weeks.[59] Once animals reach the preadult stage, the best production yields are obtained when gradually switching from a transparency-controlled food distribution (see above) to a feeding scheme of about 10% dry weight feed to live weight *Artemia* per day, distributed on a semicontinuous basis.[62] Since *Artemia* is a continuous filter-feeding organism, the highest growth and minimal deposition of unconsumed food is achieved when food is distributed as frequently as possible. The food suspension is made up daily and kept in an aerated cylindroconical tank; leftovers are discarded. The feed suspension is distributed to the culture tanks via a timer-controlled pump (Figure 6).

Culture Techniques

Depending on the objectives and opportunities, different culture procedures for super-intensive *Artemia* production may be applied. The final selection of a particular type of

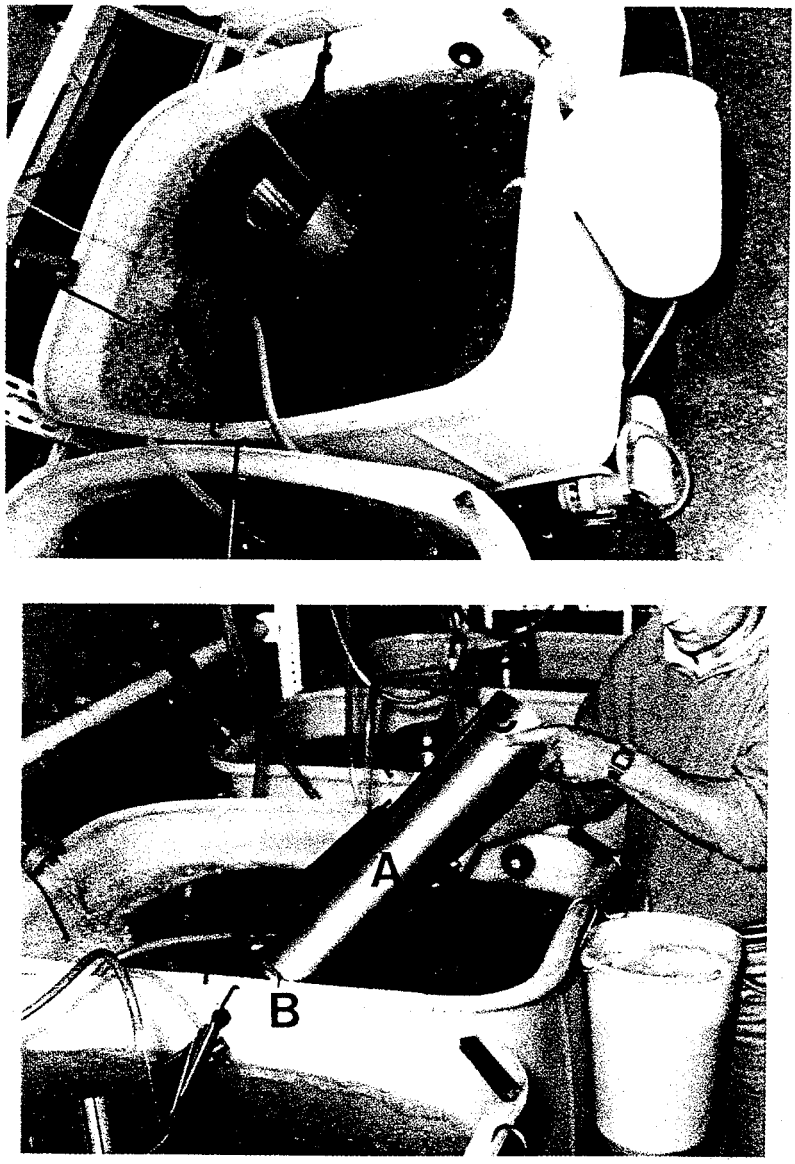

FIGURE 5. Three views of the inside of a 300-l *Artemia* culture tank equipped with standpipe and aeration collar for superintensive flow-through culturing. (A) Stainless steel welded-wedge screen cylinder; (B) aeration collar; (C) upper PVC ring.

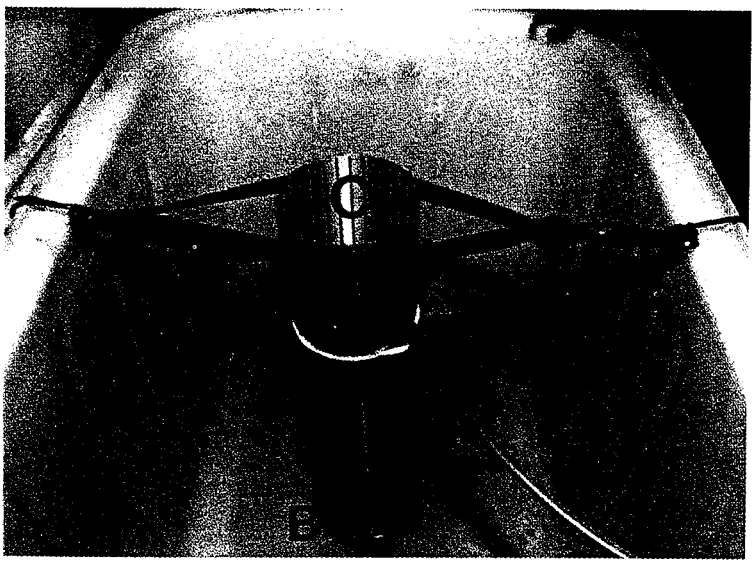

FIGURE 5 (Continued).

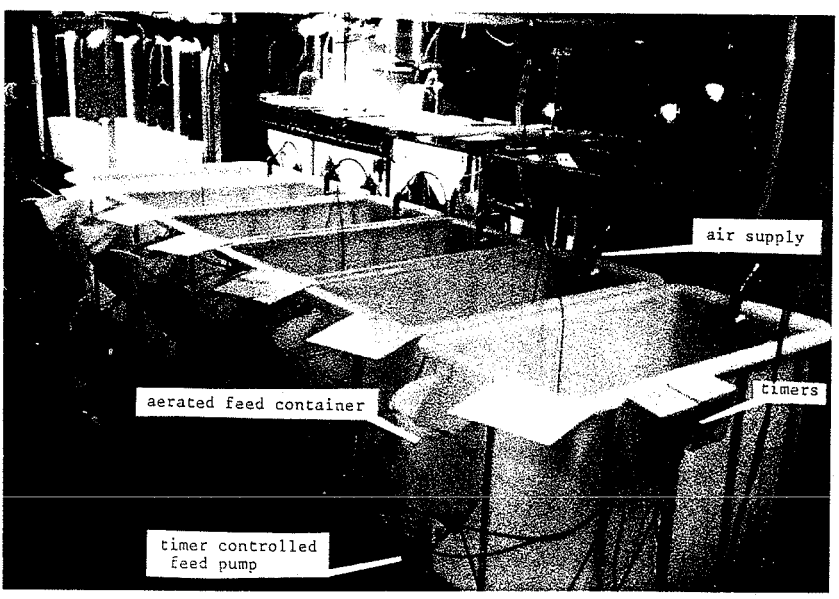

FIGURE 6. Overview of 500-l *Artemia* culture tanks for intensive batch culture.

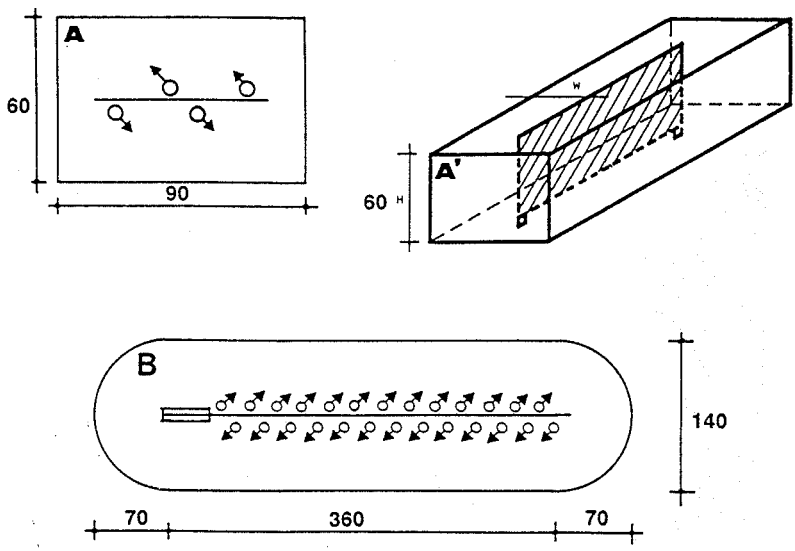

FIGURE 7. Schematic views and dimensions of raceway systems for *Artemia* culturing. (A) 300-l tank: diameter AWL 4 cm, ±7 l air/min/AWL; (B) 5 m³ tank: 80 cm water depth, diameter AWL 5 cm, ±16 l air/min/AWL. (Modified from Bossuyt, E. and Sorgeloos, P., in *The Brine Shrimp Artemia*, Vol. 3, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 133.

installation will be subject to local conditions, production needs, and investment possibilities. Two basic options are (1) should water be renewed (open flow-through) and, if not, (2) should a particular water treatment be applied (closed flow-through) or not (stagnant or batch system). Obviously, there are all kinds of transition types, ranging from open flow-through with 0% recirculation to closed flow-through with 100% recirculation. In reality, even at complete recirculation, part of the culture water must be regularly renewed.

The culture system should be designed in such a way that the water quality can be maintained as optimal as possible. This means that the concentration of particles and soluble metabolites should remain minimal in order to prevent toxicity problems, proliferation of microorganisms, and interference with the filter-feeding system of the brine shrimp.

Open Flow-Through

It is obvious that a discontinuous or continuous renewal of culture water by clean seawater, with consequent dilution of particulate and dissolved metabolites, will result in the best possible culture conditions and highest production capacities. Application of an open flow-through culture technique, however, is limited to those situations where large volumes of sufficiently warm seawater (or brine) are available at relatively low cost and/or where large quantities of algal food are available, e.g., effluents from artificial upwelling projects, tertiary treatment systems, and intensive growout ponds of shrimp. If those conditions are not fulfilled, an extra recirculation system is indispensable.

The most important and critical equipment in flow-through culturing is the special filter system used for efficient evacuation of excess culture water and metabolites without losing the brine shrimp from the culture tank. These filter units should operate without clogging for periods of more than 24 h.

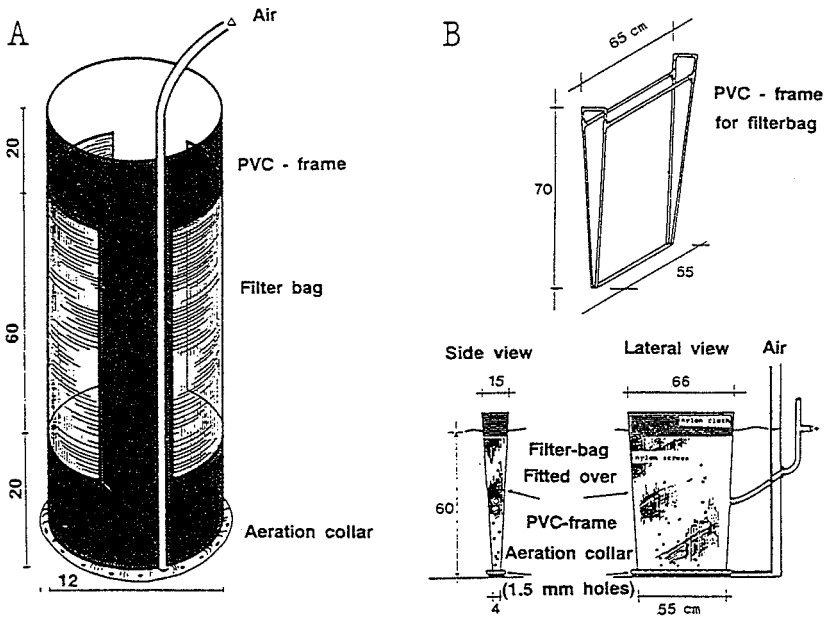

FIGURE 8. Schematic views and dimensions of filter systems used in flow-through culturing of *Artemia*. (Modified from Brisset, P. J., Versichele, D., Bossuyt, E., De Ruyck, L., and Sorgeloos, P., *Aquacult. Eng.*, 1, 115, 1982; Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, State University of Ghent, Belgium, 1986.)

Initially, filters were constructed in PVC frames around which an interchangeable nylon screen was fixed.[60,63] The aeration is positioned at the bottom of the filter and ensures a continuous friction of a curtain of air bubbles against the sides of the filter screen, which results in an efficient reduction of filter-mesh clogging (Figure 8).[60,64] The upper part of the filter bag, which is positioned just above and underneath the water level, is made of smooth nylon cloth to prevent any trapping of the cultured larvae that are foamed off by the effect of the strong aeration.

A new type of cylindrical filter system (Figure 9) was later introduced.[59] It consists of a welded-wedge screen cylinder made of stainless steel and vertically placed in the center of the culture tank (Figure 5). The base is closed by a PVC ring and bears a flexible tube for evacuation of the effluent. An aeration collar is fixed to the lower end of the filter. This welded-wedge system has several advantages over the filter-screen types:

1. Oversized particles with an elongated shape can still be evacuated through the slit openings.
2. The V-shape of the slit openings creates specific hydrodynamic effects which result in filter particles only slightly smaller than the slit opening itself being sucked through the opening.
3. Possible contact points between particles and filter are reduced to two instead of four mesh borders, which reduces the probability of clogging.

This new filter can be operated autonomously for much longer periods than with traditional mesh filters. Therefore, proportionally smaller welded-wedge filters can be used,

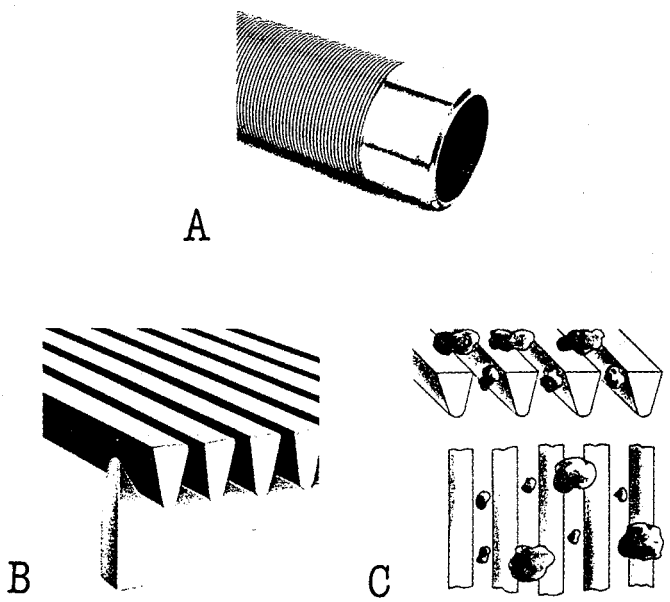

FIGURE 9. Details of a stainless welded-wedge filter used in flow-through *Artemia* culturing. (A) Overview of a filter; (B) V-profiles welded to a transverse piece; (C) Schematic presentation of the filtering mechanism.

leaving more volume for the animals in the culture tank. Furthermore, they are cost effective since they do not wear out.

As brine shrimp grow, the filters are progressively replaced by filters with a larger mesh or slit opening in order to achieve a maximal evacuation of molts, feces, and other waste particles from the culture tanks. A set of filters covering a 14-d culture period should consist of approximately six different slit/mesh openings, starting with 120 μm and progressing to 350 μm (Table 7).

The hydraulic retention time needs to be adjusted as a function of the filter size and the overall culture conditions, so as to reach an optimal compromise between efficient evacuation of wastes and minimal food losses. An optimal regime applied for a 300-l rectangular tank equipped with welded-wedge filters, 65 cm high and 14 cm in diameter, is outlined in Table 7. The flow rate can be established easily by using interchangeable PVC caps which fit onto the PVC water supply pipe connected to a constant-head tower (Figure 10). When calibrated, a series of caps with an increasing number of perforations enables the operator to maintain a preset flow rate.

A very simple semi-flow-through system has been developed by Dhert et al.[65] The system does not require the use of feeding pumps and involves minimal care. The pilot system consists of six oval 1-m^3 raceway tanks (see above) and six reservoir tanks of the same capacity placed above each culture tank (Figure 11). The reservoir tanks hold seawater and food (squeezed rice bran suspension), and need manual refilling once or twice a day. They are slowly drained to the culture tanks; the flow rate is adjusted easily by means of a siphon of a selected diameter. Retention time is at least 12 h. The culture effluent is drained

TABLE 7
Typical Example of Rate of Exchange of Filters, Culture Water Renewal, and Feeding Regime Applied in a 300-l Superintensive *Artemia* Culture

Culture day	Slit opening of filter (μm)	Flow rate (l/h)	Retention time in culture tank (h)	Interval between feeding (min)	Daily food amount (g)
1	120	80	3.75	36	100
2	150	100	3	30	120
3–4	200	100	3	24	150
5–7	250	150	2	20	180
8–9	300	150	2	20	180
10–12	350	200	1.5	15	250
13–14	350	300	1	12	300

Data compiled from Lavens, P., Ph.D. thesis, State University of Ghent, Belgium, 1989.

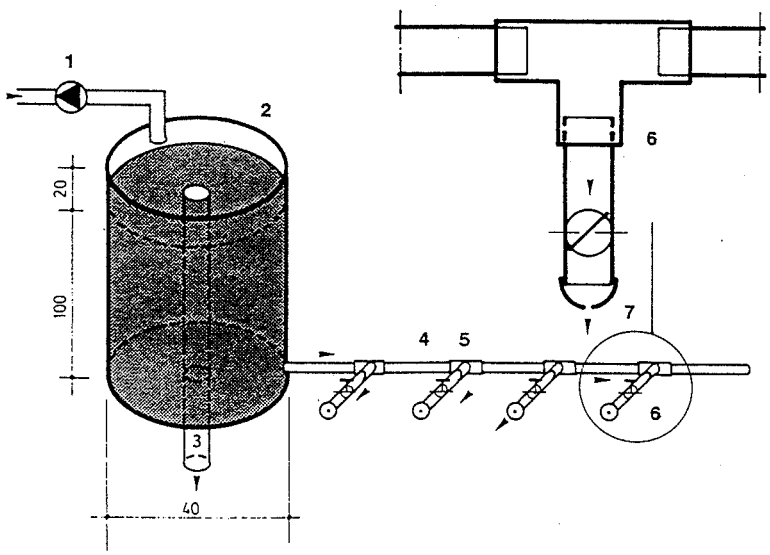

FIGURE 10. Schematic view of a water inlet system assuming precise control of the flow rates to the culture tanks. (1) Water pump; (2) constant-head tank with (3) overflow; (4) supply pipe with (5) taps; (6) PVC caps with (7) calibrated outlet opening. (Modified from Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, State University of Ghent, Belgium, 1986.)

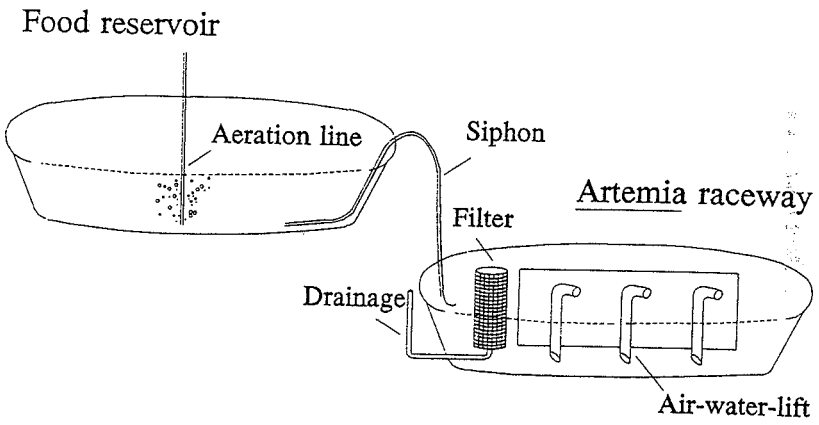

FIGURE 11. Schematic view of a 1000-l open flow-through culture system. (From Dhert, P., Bombeo, R. F., and Lavens, P., *Aquacult. Eng.*, 11, 107, 1992. With permission.)

using the welded-wedge filters described above. This technique involves minimal sophistication and appears to be very predictable in production yields, which are between those obtained in batch and flow-through systems (see Production Yields).

Closed Flow-Through Systems

When only limited quantities of warm seawater are available, open flow-through systems cannot be considered. Yet, if one decides to culture at high animal densities and/or for prolonged culture periods in batch culture, the accumulation of particles and soluble metabolites will reduce the water quality so badly that proper culture practices will become impossible. Under these conditions, the high-density flow-through culturing of *Artemia* can be maintained only by setting up a recirculation unit.

Notwithstanding much research effort to develop performing recirculation systems, the setup of a predictable culture system for marine organisms in closed circuits today is still more an art than a science. The following description is an example of an operational recirculating system for *Artemia* culture effluent, as developed at the *Artemia* Reference Center (ARC)[59] (Figure 12).

The effluent is drained from the culture tanks using filters as described above. The largest flocs are removed from the effluent in a small settling tank. Then the effluent is subjected to a biological treatment. In a rotating biological contactor (RBC), nitrogen compounds in soluble organic products are broken down via oxidative deamination, and nitrification into nitrate. Biodisc purification has been selected for our pilot installation because of its stable operation under conditions of fluctuating hydraulic and organic loadings. It consists of sand-blasted PVC discs (about 100 cm) which rotate at 6 rpm. The total effective surface for bacterial growth is 190 m^2, which is more than sufficient for the purification of 1800 l of culture water with an organic load of about 30 mg/l biological O_2 demand measured over 5 d (BOD_5).

The biodisc effluent is pumped over a cross-flow sieve[61] (200 cm^2) with a slit opening of 150 μm (Figure 13). The concentrated sludge is subsequently drained to a plate separator (Figure 14), while the purified water is returned directly to the reservoir. In this way, the volume of the plate separator can be reduced to 650 l. Its construction is a modification of the plate separator developed by Mock et al.[66] and Bossuyt and Sorgeloos.[61] It consists of

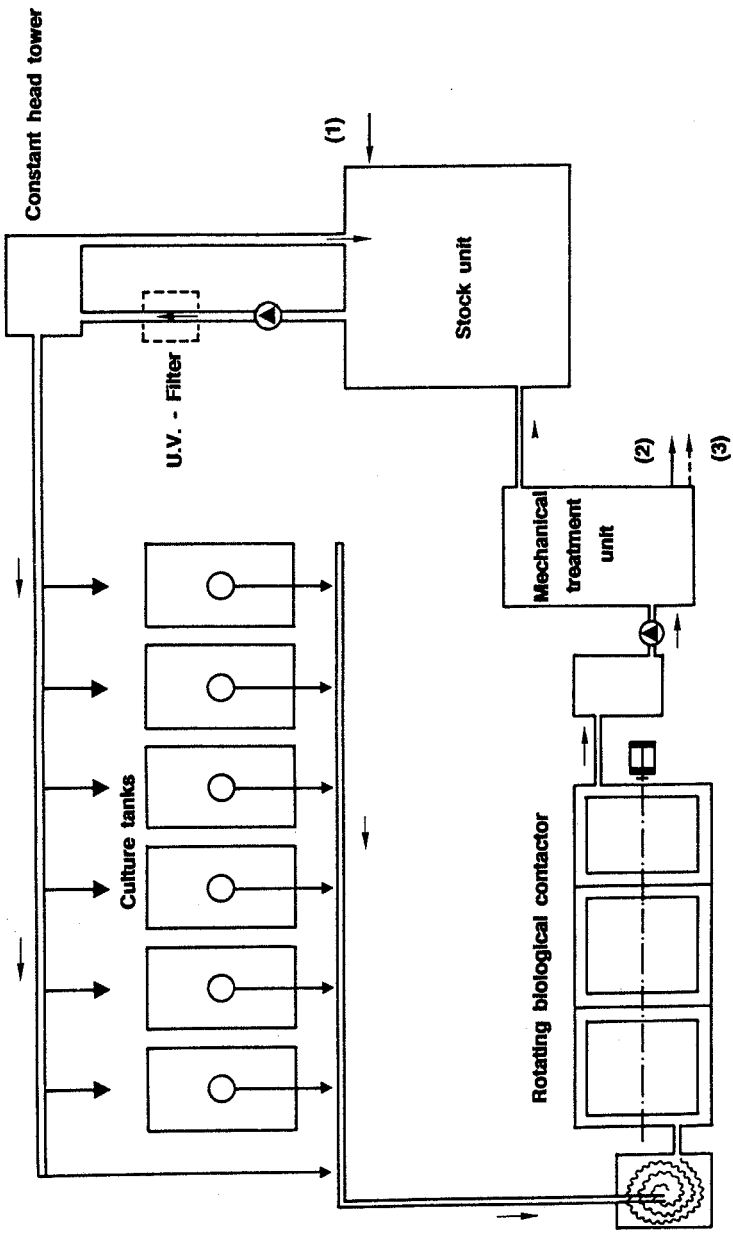

FIGURE 12. Schematic diagram of setup for closed flow-through culturing of *Artemia*. (1) Fresh seawater addition; (2) drain of water and (3) suspended solids. (From Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E., and Sorgeloos, P., *J. World Mariculr. Soc.*, 16, 498, 1986. With permission.)

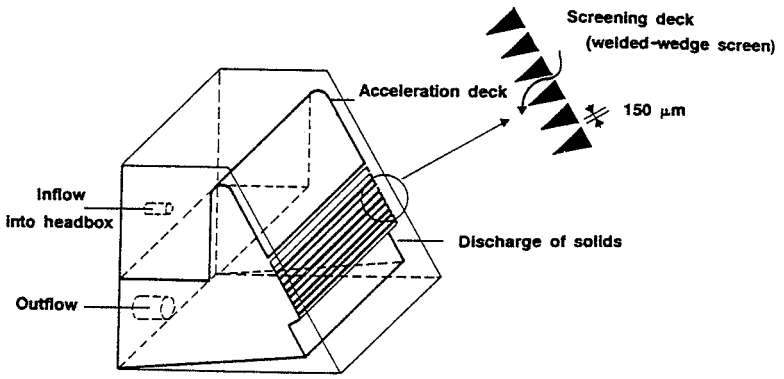

FIGURE 13. Cross-flow sieve system for concentrating suspended particles from *Artemia* culture effluents. (Modified from Bossuyt, E. and Sorgeloos, P., in *The Brine Shrimp Artemia*, Vol. 3, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 133, 1980.)

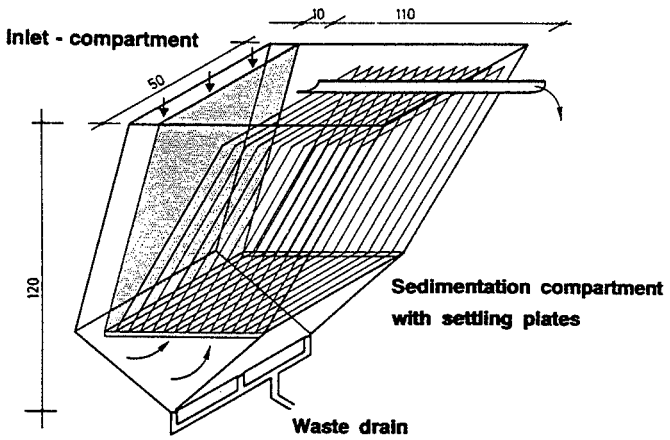

FIGURE 14. Schematic view and dimensions (in cm) of plate separator (650 l) used for primary treatment of 2-m³ *Artemia* raceway. (Modified from Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, State University of Ghent, Belgium, 1986.)

an inclined tank subdivided into a small inflow and a large settling compartment where sand-blasted PVC plates are mounted in an inclined position (60°). A drain fixed on top of the plates finally evacuates the clean water to the stock tank. Optimal retention times of about 15 min ensure maximal sedimentation of the waste particles. Once every 3 d, the accumulated sludge should be drained by siphoning from the bottom of the settling tank.

A last step in the purification process may be disinfection by ultraviolet (UV) radiation, but there is no absolute proof of its effectiveness in the culture of *Artemia* (or other organisms), nor does it result in a completely sterile medium.[67]

The treated water is pumped to a constant-head tower and returned to the culture tanks (Figure 13). At regular time intervals, part of the water should be renewed. In our system, we apply an arbitrarily chosen 25% water exchange on a weekly basis.

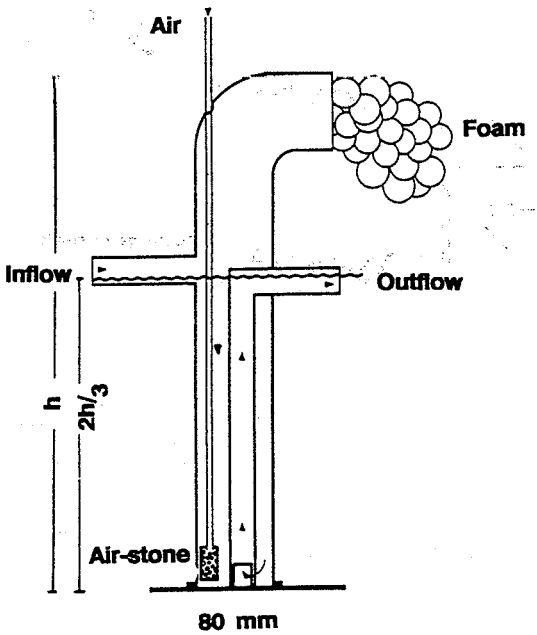

FIGURE 15. Schematic diagram of foam separator. (From Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W., and Versichele, D., *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, State University of Ghent, Belgium, 1986.

Using this recirculation system for an *Artemia* culture unit of six 300-l tanks, water quality can be maintained at acceptable levels, i.e., the biological and mechanical treatments result in an effluent containing less than 5 ml/l BOD_5[59] and 22.5 mg/l total organic carbon,[41] which accounts for a removal of more than 80%. The levels for NH_4^+-N and NO_2^--N remain below 10.9 and 0.6 ppm, respectively, which is well below the tolerance limits for *Artemia*[41] (see above). Levels for suspended solids do not exceed 380 mg/l.

The required complexity of a recirculation unit is determined by specific culture conditions such as animal density, culture duration, feed quality, tank design (sedimentation), etc. For less intensive cultures or shorter culture periods, the removal of particles with a plate separator might be sufficient, as was demonstrated by Bossuyt and Sorgeloos[61] in a 2-m^3 raceway system at 5000 animals per liter for a period of 14 d. Flow rates and plate separator dimensions are a function of the volume of the raceway; in this particular case, optimal particle removal was ensured by passing the culture medium over the plate separator at least eight times a day.

Besides the elimination of particulate wastes, it may also be desirable to partially remove the soluble fraction from the culture medium, especially when the feeding products are rich in proteins or contain a high fraction of soluble material. Bossuyt and Sorgeloos successfully used foam fractioning[68] for the final treatment of the effluent of a 2-m^3 raceway (Figure 15).

Stagnant Systems

Although the advantages of using prey with a gradually increasing size are fully recognized, the complexity and additional costs of growing *Artemia*, compared to nauplii,

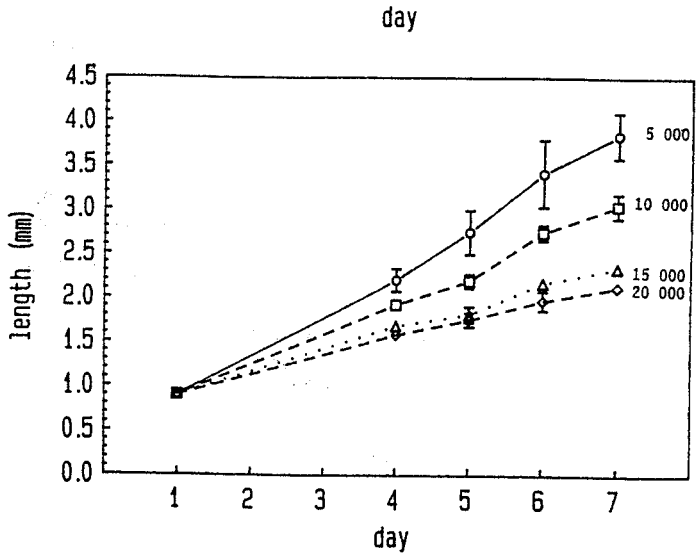

FIGURE 16. Growth curves of *Artemia* cultured under batch conditions at 5 animals/ml (○), 10 animals/ml (□), 15 animals/ml (△), and 20 animals/ml (◇).

remains prohibitive for the generalized use of ongrown *Artemia*. Therefore, a simplified but reliable technique for the short-term intensive culture of *Artemia* juveniles for use as a nursery diet for fish and shrimp is being developed at the ARC. The idea is to develop a flexible culture procedure covering the production of *Artemia* of various sizes up to 3 mm, and improving their nutritional value through simple enrichment techniques. In fact, higher HUFA levels are obtained than for nauplii enrichment.

Cultures were conducted in 100- or 500-l rectangular polyethylene tanks. Natural filtered seawater at 25°C was used, and no water renewal was applied. Aeration is ensured by four perforated PVC tubes fixed to the bottom. GSL cysts are hatched, counted, and transferred to the culture tanks at 5, 10, 15, 20, or 50 animals per milliliter, depending on the desired growth rate (Figure 16). The culture period was arbitrarily confined to 7 d. The animals were fed YM20 (*Artemia* Systems N.V., Belgium), a micronized mixture of peas and corn waste. The feed was mixed daily in tap water and distributed semicontinuously to the culture using a peristaltic pump or a timer-controlled submersible pump. Daily feed ratios were chosen so as to keep the transparency of the culture water between 15 and 20 cm. Although a planned feeding regime was established (Table 8), each culture required constant adjustment of the ratios according to the observed transparency.

Artemia juveniles can be enriched in the same way as nauplii, resulting in higher HUFA levels in shorter enrichment periods (Table 9). There is, however, an alternative method that takes advantage of the specific aspects of ongrown *Artemia:* instead of adding the enrichment emulsion at the end of the culture, the emulsion is distributed during the culture.[69] Daily increasing doses of DRY SELCO (*Artemia* Systems N.V., Belgium) were added to the food suspension so that the total DRY SELCO ratio equaled 0.6 g/l by the end of the culture (Table 8). This method has several advantages: (1) HUFA levels can be five times higher than when the same amount is given in one ration after 7 d (Table 9), (2) the risk

TABLE 8
Directive Feedings and Enrichment Regime for the Stagnant Production System (g/100 l Culture Volume)

	Day							
	0	1	2	3	4	5	6	Total
YM20	200	225	250	300	325	350	350	2000
DRY SELCO	—	37.5	62.5	87.5	112.5	137.5	162.5	600

Note: Density, 10/ml; feed, YM20, Artemia Systems, Belgium; booster, DRY SELCO, Artemia Systems, Belgium.

Data compiled from Dhont, J., Lavens, P., and Sorgeloos, P., in *Book of Abstracts, Larvi '91 — Fish & Crustacean, Larviculture Symposium*, Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F., Eds., Spec. Publ. No. 15, European Aquaculture Society, Ghent, Belgium, 1991, 51.

TABLE 9
Comparison of Enrichment Results of *Artemia* Juveniles and Nauplii

Artemia	Procedure	Duration	20:5n-3 (mg/g DW)	22:6n-3 (mg/g DW)	Σ n-3 HUFA (mg/g DW)
Nauplii[a]	After hatching	12 h	7.9	4.4	14.4
Juveniles[b]	After culture	4 h	5.8	4.4	14.2
	During culture	7 d	44.2	16.5	64.3

[a] Data compiled from Dhont, et al.[69]
[b] Data compiled from Léger, et al.[33]

of oxygen drops is avoided, and (3) *Artemia* that are harvested before the end of the culture are already partially enriched.

Production yields are presented in the section titled "Production Yields".

Control of Infections

When experimenting with micronized agricultural byproducts as feed for *Artemia* cultures, we occasionally faced heavy losses of preadults due to infections with filamentous *Leucothrix* bacteria.[41,59] According to Solangi et al.,[70] these infections occur especially in nutrient-rich media. The *Leucothrix* colonies fix on the exoskeleton, by preference on the thoracopods, and are only visible from the instar V/VI stage onward. The brine shrimp suffer physically, as the movements of their thoracopods become affected, and hence the filtration rates are reduced. At the maximum limit of infection, growth and molting are arrested, and overfeeding of the tanks occurs, eventually resulting in the collapse of the *Artemia* culture.

Solangi et al. recommend the application of terramycin;[70] however, antibiotics cannot be used in recirculation systems as they will affect the biological treatment unit. The most practical solution appears to be to raise the salinity from 35 to 50 or 60 ppt, together with a higher water renewal rate of 25% instead of 10% on a weekly basis.[41,59]

A second observed disease in *Artemia* cultures is the so-called "black disease", i.e., some of the animals show black spots, especially at their extremities (e.g., thoracopods, antennae). According to Hernandorena,[71,72] this disease consists of the detachment of the

epidermis from the cuticula, and is caused by a dietary deficiency which interferes with the lipid metabolism. In high-density culturing of *Artemia* using agricultural byproducts as a food source, we observe the black disease when water quality deteriorates (probably interfering with the composition of the bacterial population and, consequently, the composition of the diet) and/or when feeding rates are not optimal. Improving these conditions does not save the affected animals, but does appear to avoid further losses.

Harvesting and Processing Techniques

Harvesting of high-density cultures of *Artemia* can be facilitated by taking advantage of the surface respiration behavior of the animals. When the aeration in the culture tank together with the flow-through and the automatic feeding are interrupted, oxygen levels in the water drop very fast and all waste particles sink to the bottom; after about 30 min, the *Artemia* respond to the oxygen depletion by concentrating at the water surface, where they perform surface respiration. The concentrated population, free from suspended solids, can easily be scooped out with a net of appropriate mesh size.

When the culture water is not loaded with particles, brine shrimp can be harvested by draining the complete culture over a sieve, which should be partially submerged.

After their thorough wash in fresh water or seawater, the harvested *Artemia* can be offered as a most suitable live food to freshwater and marine predators. The salinity of the culture water is of no concern, as *Artemia* is a hypo-osmoregulator, i.e., its body fluids have a constant and low salt content of about 9 ppt, which renders it an acceptable food for freshwater animals as well. Furthermore, when transferred into fresh water, *Artemia* will continue to swim for another 5 h, after which time they eventually die as a result of osmoregulatory stress. In seawater, they remain alive without feeding for several days. Transportation of live brine shrimp can be done in plastic bags containing cooled seawater and inflated with oxygen.

Harvested *Artemia* which are not directly consumed can be frozen or dried in flakes after a thorough washing with fresh water. In order to ensure optimal product quality, brine shrimp biomass must be frozen when still alive. The biomass should be spread out in thin layers (1 cm) in plastic bags or in ice trays and be transferred to a quick freezer (at least −25°C). The adult exoskeleton is not damaged when the biomass is properly frozen. Upon thawing, the *Artemia* cubes yield intact animals which do not pollute the water by leaching body fluids.

Production Yields

Figure 17 provides a summary of average production data expressed in terms of the *Artemia* survival and length obtained in the different culture systems described in this chapter. After 2 weeks of culturing, preadult or adult *Artemia* with an average length of 5 mm or more can be harvested. Under flow-through culture conditions, significant mortality often occurs in the first week, probably because the early naupliar stages are more sensitive than the juvenile or preadult stages. No significant differences in survival are noticed between open and closed (with recirculation of the culture medium) flow-through cultures. On the other hand, in stagnant culture installations survival decreases continuously into the second week, which can be explained by the deterioration of the water quality.

Average production yields in terms of wet weight biomass harvested after 2 weeks of culture amount to 5, 15, and 25 kg of *Artemia* per cubic meter of tank volume for batch production,[58] flow-through systems using micronized feeds,[59,64] and live algae, respectively.[60] These differences in production figures are mainly the result of differences in maximum stocking density at the start and of final survival at the end of the culture trial.

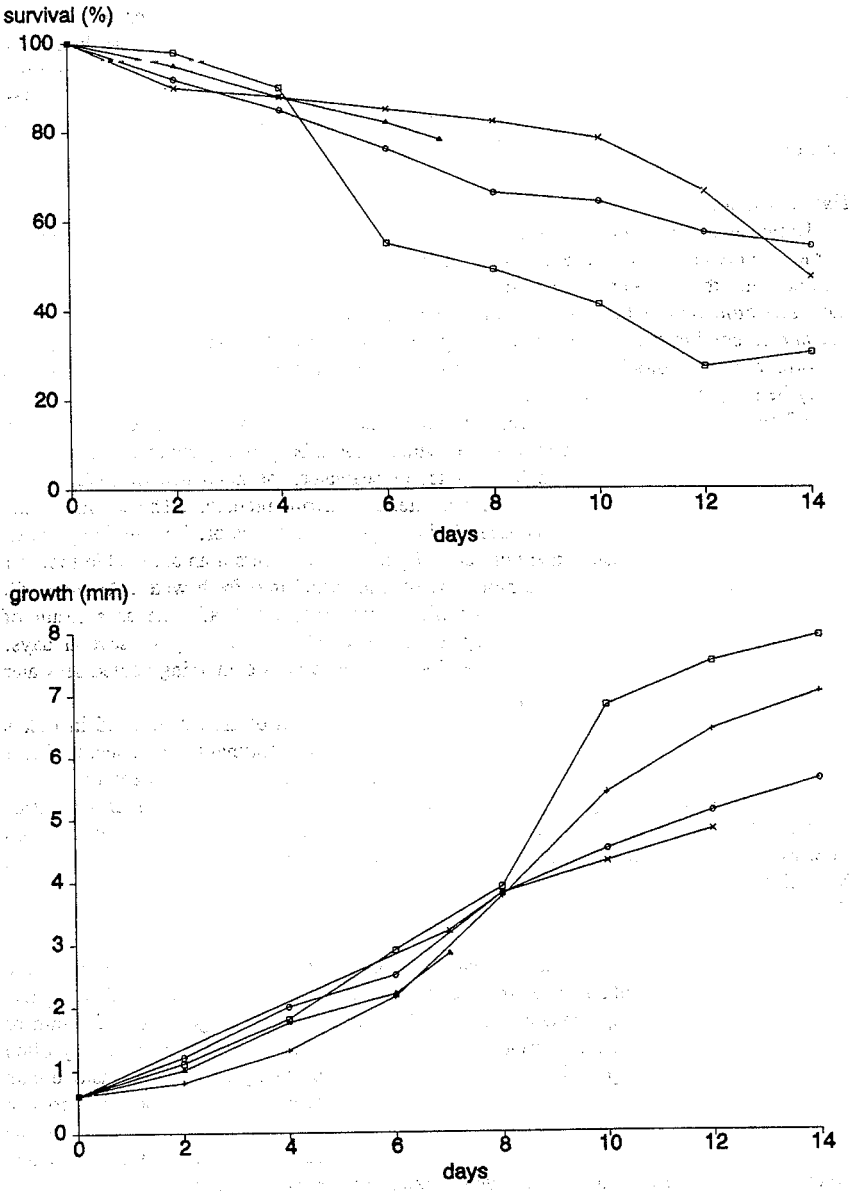

FIGURE 17. Survival (top) and growth (bottom) of Great Salt Lake *Artemia* cultured in different superintensive culture systems. —×—, Open flow-through culture (1000-l) on rice bran diet. (From Dhert, P., Bombeo, R. F., and Lavens, P., *Aquacult. Eng.*, 11, 107, 1992.) —+—, Open flow-through culture (200-l) on *Chaetoceras* diet. (Modified from Lavens, P., Ph.D. thesis, State University of Ghent, Belgium, 1989.) —○—, Closed flow-through culture (300-l) on a mixture of soybean waste and corn bran. (Modified from Lavens, P., Ph.D. thesis, State University of Ghent, Belgium, 1989.) —□—, Closed flow-through raceway culture (2 m^3) on rice bran diet. (Modified from Lavens, P., Ph.D. thesis, State University of Ghent, Belgium, 1989). —△—, Batch culture (500-l) on a mixture of pea and corn bran. (From Dhont, J., in preparation.)

TABLE 10
Comparison of the Biochemical Composition of Nauplii and Preadults Harvested from Superintensive Cultures

Artemia characteristics	Proteins	Lipids	N-free extract	Ash	Ref.
Instar I — nauplii	41.6–47.2	20.8–23.1	10.5–22.79	.5	89–91
Preadults cultured on corn bran/soybean pellets in flow-through system	55.2	17.6	10.4	16.8	76
Preadults cultured on rice bran in stagnant raceway system	56.5	19.5	—	9.0	92

Note: All data are for the Great Salt Lake strain and are expressed as percent of dry weight.

THE NUTRITIONAL VALUE OF ONGROWN *ARTEMIA*

The nutritional quality of *Artemia* juveniles and adults produced in superintensive systems is analogous to the natural product, except for its lipid content. Compared to instar-I nauplii, the protein content of adult *Artemia*, independent of its rearing conditions or food, is appreciably higher, i.e., 60% instead of 47%, and especially richer in essential amino acids[73] (Tables 10 and 11). On the other hand, the lipid profile is a reflection of the diet offered to the *Artemia* cultures (Table 11). This does not necessarily restrict their application, since high levels of EFAs can be attained easily in the *Artemia* biomass by applying the simple bioencapsulation techniques described above.

Artemia juveniles and adults are used as a nursery diet, not only for their optimal nutritional value, but for energetic advantages as well, i.e., when offered large *Artemia* instead of freshly hatched nauplii, the predator larvae need to chase fewer prey organisms per unit of time to meet their food requirements. This improved energy balance may result in better growth, faster developmental rate, and/or improved physiological condition.[65,74-76] Moreover, the introduction of ongrown *Artemia* as a hatchery/nursery food may result in significant savings of *Artemia* cysts and, consequently, in a substantial reduction of the total larval feed cost.

Ongrown *Artemia* have been used successfully for postlarval feeding of *Penaeus monodon*,[77,78] *P. kerathurus*,[79,80] *P. japonicus*,[81-83] *P. aztecus*,[84] *P. vanamei*,[85] and *Macrobrachium rosenbergii*.[86] Yashiro[78] compared growth and survival in *P. monodon* postlarvae reared on *Artemia* of progressively larger size that were fed diets of wheat flour, rice bran, and milled rice. Freshly hatched *Artemia* supplemented with cooked mussel meat were fed in the control treatment. Growth after 20 d was significantly better in the treatments fed ongrown *Artemia* (more than double the weight gain), while survival was best in the control treatment, though not significantly better than those shrimp fed *Artemia* grown on milled rice.

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TABLE 11
Fatty Acid and Amino Acid Profiles of Great Salt Lake Preadults Cultured under Flow-Through Conditions

FATTY ACIDS[a]		AMINO ACIDS[b]	
Saturated	16.20	Essential	26.94
14:0	0.70	tryptophan	
15:0	0.50	lysine	4.23
16:0	9.10	histidine	1.30
17:0	0.70	arginine	2.69
18:0	5.20	threonine	2.42
19:0	—	valine	3.20
20:0	—	methionine	0.71
24:0	—	isoleuoine	2.96
Unsaturated	46.70	leucine	4.52
14:1	1.20	tyrosine	2.16
14:2	—	phenylalaline	2.75
15:1	0.30	Nonessential	25.71
16:1n-7 + 16:1n-9	4.30	asparagine	5.82
16:2	—	serine	2.63
16:3	0.30	glutamine	7.64
17:1	—	proline	3.29
18:1n-7 + 18:1n-9	18.30	glycine	2.68
18:2	15.90	alanine	3.61
18:4	0.10	cysteine	0.14
19:4	—		
20:1	4.00		
21:5	0.30		
22:1	2.00		
24:1			
Unsaturated (n-3)	9.00		
18:3	—		
20:3	0.30		
20:4	—		
20:5	2.80		
22:3	0.90		
22:4	—		
22:5	0.40		
22:6	4.60		
Unsaturated (n-6)	0.40		
18:3	—		
20:3	—		
20:4	—		
22:4	0.40		
22:5	—		

Note: Data compiled from Abelin, P., personal communication, 1988. Diet was mixture of corn bran and soybean pellets.

[a] Values expressed in mg/g dry matter.
[b] Values expressed in g/100 g dry matter.

REFERENCES

1. **Seale, A.,** Brine shrimp (*Artemia*) as a satisfactory food for fishes, *Trans. Am. Fish. Soc.*, 63, 129, 1933.
2. **Gross, F.,** Notes on the culture of some marine plankton organisms, *J. Mar. Biol. Assoc. U.K.*, 21, 753, 1937.
3. **Rollefson, G.,** Artificial rearing of fry of seawater fish — preliminary communication, *Rapp. P. Reun. Cons. Perm. Int. Explor. Mer.*, 109, 133, 1939.
4. **Sorgeloos, P.,** Live animal food for larval rearing in aquaculture: the brine shrimp *Artemia*, in *Realism in Aquaculture: Achievements, Constraints, Perspectives*, Bilio, M., Rosenthal, H., and Sinderman, C. J., Eds., European Aquaculture Society, Bredene, Belgium, 1986, 199.
5. **Sorgeloos, P., Persoone, G., Baeza-Mesa, M., Bossuyt, E., and Bruggeman, E.,** The use of *Artemia* cysts in aquaculture: the concept of "hatching efficiency" and description of a new method for cyst processing, in Proc. 9th Annu. Meet. World Mariculture Society, 1978, 715.
6. **Vanhaecke, P. and Sorgeloos, P.,** International Study on *Artemia*. XIX. Hatching data on 10 commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept, *Aquaculture*, 30, 43, 1983.
7. **Vanhaecke, P. and Sorgeloos, P.,** International Study on *Artemia*. XVIII. The hatching rate of *Artemia* cysts — a comparative study, *Aquacult. Eng.*, 1(4), 263, 1982.
8. **Benijts, F., Vanvoorden, E., and Sorgeloos, P.,** Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina*, 10th Eur. Symp. Marine Biology, Ostend, Belgium, 1976, 1.
9. **Lavens, P. and Sorgeloos, P.,** The cryptobiotic state of *Artemia* cysts, its diapause deactivation and hatching: a review, in *Artemia Research and its Application*, Vol. 3, Ecology, Culturing, Use in Aquaculture, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 27.
10. **Crowe, J. H. and Clegg, J. S.,** *Anhydrobiosis*, Dowden, Hutchinson and Ross, Stroudsberg, PA, 1973, 477.
11. **Kinne, O.,** Research cultivation, in *Marine Ecology*, Vol. 3, Kinne, O., Ed., Interscience, New York, 1977, 579.
12. **Léger, Ph., Bengtson, D. A., Simpson, K. L., and Sorgeloos, P.,** The use and nutritional value of *Artemia* as a food source, in *Oceanography and Marine Biology. An Annual Review*, Vol. 24, Aberdeen University Press, Aberdeen, U.K., Barnes, M., Ed., 1986, 521.
13. **Lavens, P., Léger, Ph., and Sorgeloos, P.,** Manipulation of the fatty acid profile in *Artemia* offspring using a controlled production unit, in *Aquaculture — A Biotechnology in Progress*, De Pauw, N., Jaspers, E., Ackefors, H., and Wilkins, N., Eds., European Aquaculture Society, Bredene, Belgium, 1989, 731.
14. **Seidel, C. R., Kryznowek, J., and Simpson, K. L.,** International Study on *Artemia*. XI. Amino acid composition and electrophoretic patterns of *Artemia* from five geographical locations, in *The Brine Shrimp Artemia*, Vol. 3, *Ecology — Culturing — Use in Aquaculture*, Personne, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 375.
15. **Dabrowski, K. and Rusiecki, M.,** Contents of total and free amino acids in zooplanktonic food of fish larvae, *Aquaculture*, 30, 31, 1983.
16. **Ezquieta, B. and Vallejo, C. G.,** *Artemia* trypsin-like proteinase — an developmentally regulated proteinase, in *Artemia Research and its Application*, Vol. 2, *Physiology, Biochemistry, Molecular Biology*, Decleir, W., Moens, L., Slegers, H., Sorgeloos, P., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 53.
17. **Perona, R., Ezqueito, B., and Vallejo, C. B.,** The degradation of yolk in *Artemia*, in *Artemia Research and its Application*, Vol. 2, *Physiology, Biochemistry, Molecular Biology*, Decleir, W., Moens, L., Slegers, H., Sorgeloos, P., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 105.
18. **Dabrowski, K. and Glogowski, J.,** Studies on proteolytic enzymes of invertebrates constituting fish food, *Hydrobiologia*, 52(2/3), 171, 1977.
19. **Cowgill, U. M., Emmel, H. W., Boggs, G. U., Murphy, P. G., Gersich, F. M., Takahashi, I. T., and Bengtson, D. A.,** Variations in chemical composition of *Artemia* cysts from three geographical locations, in *Artemia Research and Its Application*, Vol. 1, *Morphology, Genetics, Strain Characterization, Toxicology*, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 173.
20. **Wheeler, R., Yudin, A. I., and Clark, W. H., Jr.,** Hatching events in the cysts of *Artemia salina*, *Aquaculture*, 18, 59, 1979.
21. **Sorgeloos, P., Lavens, P., Leger, Ph., Tackaert, W., and Versichele, D.,** *Manual for the Culture and Use of Brine Shrimp Artemia in Aquaculture*, manual prepared for the Belgian Administration for Development Cooperation and the Food and Agriculture Organization of the United Nations, *Artemia* Reference Center, Faculty of Agriculture, State University of Ghent, Belgium, 1986, 319.

22. **Sorgeloos, P.,** First report on the triggering effect of light on the hatching mechanism of *Artemia salina* dry cysts, *Mar. Biol.*, 22, 75, 1973.
23. **Clegg, J. S.,** The control of emergence and metabolism by external osmotic pressure and the role of free glycerol in developing cysts of *Artemia salina*, *J. Exp. Biol.*, 41, 879, 1964.
24. **Sorgeloos, P., Bossuyt, E., Lavina, E., Baeza-Mesa, M., and Persoone, G.,** Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture, *Aquaculture*, 12, 311, 1977.
25. **Vanhaecke, P., Lavens, P., and Sorgeloos, P.,** International study on Artemia XVII. Energy consumption in cysts and early larval stages of various geographical strains of *Artemia*. *Ann. Soc. Zool. Belg.*, 113(2), 155, 1983.
26. **Vanhaecke, P. and Sorgeloos, P.,** International study on Artemia XIX. Hatchings data for 10 commercial sources of brine shrimp, cysts, and re-evaluation of the "hatching efficiency" concept, *Aquaculture*, 30, 43, 1983.
27. **Vanhaecke, P. and Sorgeloos, P.,** International study on Artemia IV. The biometrics of *Artemia* strains from different geographical origin, in *The Brine Shrimp Artemia, Vol. 3: Ecology, Culturing, Use in Aquaculture*, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 456.
28. **Sorgeloos, P.,** Availability of reference *Artemia* cysts, *Mar. Ecol.*, 3, 363, 1980.
29. **Bengtson, D. A., Léger, Ph., and Sorgeloos, P.,** Use of *Artemia* as a food source in *Artemia Biology*, Browne, R. A., Sorgeloos, P., and Trotman, C. N. A., Eds., CRC Press, Boca Raton, FL, 1991, 255.
30. **Bruggeman, E., Sorgeloos, P., and Vanhaecke, P.,** Improvements in the decapsulation technique of *Artemia* cysts, in *The Brine Shrimp Artemia*, Vol. 3, *Ecology, Culturing, Use in Aquaculture*, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 261.
31. **Miller, D. C., Lang, W. H., Marey, M., Clem, P., and Pechenik, J.,** International Study on *Artemia*. Naupliar locomotory rates, patterns and photoresponses: a comparative study of various strains of *Artemia salina*, in Abstr. Int. Symp. Brine Shrimp *Artemia salina*, Corpus Christi, TX, 1979, 19.
32. **Léger, Ph., Vanhaecke, P., and Sorgeloos, P.,** International Study on *Artemia*. XIV. Cold storage of live *Artemia* nauplii from various geographical sources: potentials and limits in aquaculture, *Aquacult. Eng.*, 2(1), 69, 1983.
33. **Léger, Ph., Naessens-Foucquaert, E., and Sorgeloos, P.,** International Study on *Artemia*. XXXV. Techniques to manipulate the fatty-acid profile in *Artemia* nauplii, and the effect on its nutritional effectiveness for the marine crustacean Mysidopsis bahia (M.), in *Artemia Research and its Applications*, Vol. 3, *Ecology, Culturing, Use in Aquaculture*, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 411.
34. **Lai, L. and Lavens, P.,** Report of workshop: *Artemia* as a business perspective, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 517.
35. **Lavens, P. and Sorgeloos, P.,** Production of *Artemia* in culture tanks, in *Artemia Biology*, Browne, R. A., Sorgeloos, P., and Trotman, C. N. A., Eds., CRC Press, Boca Raton, FL, 1991, 255.
36. **Vanhaecke, P., Siddall, S. E., and Sorgeloos, P.,** International Study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin, *J. Exp. Mar. Biol. Ecol.*, 80, 259, 1984.
37. **Provasoli, L. and D'Agostino, A. S.,** Development of artificial media for *Artemia salina*, *Biol. Bull.*, 136, 434, 1969.
38. **McShan, M., Trieff, N. M., and Grajcer, D.,** Biological treatment of wastewater using algae and *Artemia*, *J. Water Pollut. Control Fed.*, 46, 1742, 1974.
39. **Decleir, W., Vos, J., Bernaerts, F., and Van den Branden, C.,** The respiratory physiology of *Artemia*, in *The Brine Shrimp Artemia*, Vol. 2, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 137.
40. **Platon, R. R.,** Scale-Up Studies on the Culture of Brine Shrimp *Artemia* Fed with Rice Bran, Ph.D. thesis, University of British Columbia, Vancouver, Canada, 1985, 170.
41. **Lavens, P.,** Intensieve Produktie en Kwaliteitsonderzoek van *Artemia* Adulten en hun Nakomelingen, Ph.D. thesis, State University of Ghent, Belgium, 1989.
42. **Chen, J. C., Chen, K. J., and Liao, J. M.,** Joint action of ammonia and nitrite in *Artemia salina* nauplii, in 19th Annu. Meet. World Aquaculture Society, Honolulu, HI, 1988, 26.
43. **Coutteau, P. and Sorgeloos, P.,** Feeding of the brine shrimp *Artemia* on yeast: effect of mechanical disturbance, animal density, water quality and light intensity, in *Aquaculture Europe '89, Short Communications and Abstracts*, Billard, R. and De Pauw, N., Eds., Spec. Publ. No. 10, European Aquaculture Society, Bredene, Belgium, 1989, 75.

44. **Douillet, P.,** Effect of bacteria on the nutrition of the brine shrimp *Artemia*, fed on dried diets, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 295.
45. **D'Agostino, A. S.,** The vital requirements of *Artemia:* physiology and nutrition, in *The Brine Shrimp Artemia*, Vol. 2, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 55.
46. **Sorgeloos, P.,** High density culturing of the brine shrimp, *Artemia salina*, *Aquaculture*, 1, 385, 1973.
47. **Cognie, D.,** Production d'*Artemia salina* leach en grand volumes recyclés, in *10th European Symposium on Marine Biology*. Vol. 1, Persoone, G. and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1976, 35.
48. **Person-Le Ruyet, J.,** Elevage larvaire d'*Artemia salina* (Branchipode) sur nourriture inerte: *Spirulina maxima* (Cyanophycée), *Aquaculture*, 8, 157, 1976.
49. **Paulsen, C.,** *Artemia* culture, in *Annual Report 1977 of the Oceanic Institute*, Waimanalo, HI, 1978, 11.
50. **Klein, V. L. M. and Ibarra Su, J.,** Different ration types in the feeding of *Artemia salina* in laboratory conditions, in Abstr. 1st Interam. Congr. Aquaculture, Salvador, Brazil, September 14 to 21, 1986, 68.
51. **Vandamme, E. J. and Demey, L.,** De Produkctie van "Single Cell Protein", *Hét Ingenieursblad*, 49, 89, 1980.
52. **Peppler, H. J. and Stone, C. W.,** Feed yeast products. An update of the properties of feed yeasts and their applications, *Feed Manage.*, August, 20, 1976.
53. **Litchfield, J. H.,** Microbial protein production, *Bioscience*, 30, 387, 1980.
54. **Urban, E., Jr. and Langdon, C.,** Reduction in costs of diets for the American oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements, *Aquaculture*, 38, 277, 1984.
55. **Hirayama, K.,** A consideration of why mass culture of the rotifer *Brachionus plicatilis* with baker's yeast is unstable, *Hydrobiologia*, 147, 269, 1987.
56. **Coutteau, P., Lavens, P., and Sorgeloos, P.,** *The Use of Yeast as Single-Cell Protein in Aquacultural Diets*, Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniv. Gent, 1989, 54.
57. **Dobbeleir, J., Adam, N., Bossuyt, E., Bruggeman, E., and Sorgeloos, P.,** New aspects of the use of inert diets for high density culturing of brine shrimp, in *The Brine Shrimp Artemia*, Vol. 3, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 165.
58. **Bossuyt, E. and Sorgeloos, P.,** Batch production of adult *Artemia* in 2 m^3 and 5 m^2 air-water-lift operated raceways, in *Abstr. World Conference on Aquaculture*, Venice, Italy, 1981, 17.
59. **Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E., and Sorgeloos, P.,** New developments in the high density flow-through culturing of brine shrimp *Artemia*, *J. World Maricult. Soc.*, 16, 498, 1986.
60. **Tobias, W. J., Sorgeloos, P., Bossuyt, E., and Roels, O. A.,** The technical feasibility of mass-culturing *Artemia salina* in the St. Croix "artificial upwelling" mariculture system, *Proc. World Maricult. Soc.*, 10, 203, 1979.
61. **Bossuyt, E. and Sorgeloos, P.,** Technological aspects of the batch culturing of *Artemia* in high densities, in *The Brine Shrimp Artemia*, Vol. 3, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 133.
62. **Lavens, P. and Sorgeloos, P.,** Design, operation, and potential of a culture system for the continuous production of *Artemia* nauplii, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 339.
63. **Tobias, W. J., Sorgeloos, P., Roels, O. A., and Sharfstein, B. A.,** International Study on *Artemia*. XIII. A comparison of production data of 17 geographical strains of *Artemia* in the St. Croix artificial upwelling-mariculture system, in *The Brine Shrimp Artemia*, Vol. 3, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 383.
64. **Brisset, P. J., Versichele, D., Bossuyt, E., De Ruyck, L., and Sorgeloos, P.,** High density flow-through culturing of brine shrimp *Artemia* on insert feeds — preliminary results with a modified culture system, *Aquacult. Eng.*, 1, 115, 1982.
65. **Dhert, P., Bombeo, R. F., and Lavens, P.,** Optimization of a semi flow-through system for the super-intensive culture of the brine shrimp *Artemia*, *Aquacult. Eng.*, 11, 107, 1992.
66. **Mock, C. R., Ross, L. R., and Salser, R. B.,** Design and preliminary evaluation of a closed system for the shrimp culture, in *Proc. 8th Annu. Meet. World Mariculture Society*, Avault, J. W., Jr., Ed., Louisiana State University, Baton Rouge, 1977, 335.
67. **Rosenthal, H.,** Ozonization and sterilization, in *Aquaculture in Heated Effluents and Recirculation Systems*, Vol. 1, Tiews, K., Ed., Heeneman, Berlin, 1981, 219.
68. **Wheaton, F. W.,** *Aquacultural Engineering*, Interscience, New York, 1977.
69. **Dhont, J., Lavens, P., and Sorgeloos, P.,** Development of a lipid-enrichment technique for *Artemia* juveniles produced in a super intensive system for use in marine larviculture, in *Book of Short Communications and Abstracts, Larvi '91 — Fish & Crustacean Larviculture Symposium*, Lavens, P., Sorgeloos, P., Jaspers, E., and Ollevier, F., Eds., Spec. Publ. No. 15, European Aquaculture Society, Ghent, Belgium, 1991, 51.

70. **Solangi, M. A., Overstreet, R. M., and Gannam, A. L.,** A filamentous bacterium on the brine shrimp and its control, *Gulf Res. Rep.*, 6, 275, 1979.
71. **Hernandorena, A.,** Nutrition, Croissance et Metabolisme d' *Artemia salina* (L.), Ph.D. thesis, University of Paris, France, 1974, 154.
72. **Hernandorena, A.,** An increased dietary tryptophan requirement induced by interference with purine interconversion in *Artemia*, in *Artemia Research and its Applications*, Vol. 2, Decleir, W., Moens, L., Slegers, H., Sorgeloos, P., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 233.
73. **Ronsivalli, P. C. and Simpson, K. L.,** The brine shrimp *Artemia* as a protein source for humans, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 503.
74. **Sick, L. V. and Beaty, H.,** Development of formula foods designed for *Macrobrachium rosenbergii* larval and juvenile shrimp, in Proc. 6th Annu. Meet. World Mariculture Society, Avault, J. W., Jr. and Miller, R., Eds., Louisiana State University, Baton Rouge, 1975, 557.
75. **Purdom, C. E. and Preston, A.,** A fishy business, *Nature (London)*, 266, 396, 1977.
76. **Abelin, P.,** personal communication, 1988.
77. **Hanson, J. A. and Goodwin, H. L.,** Shrimp farming in the Western Hemisphere, Dowden, Hutchinson & Ross, Stroudsburg, PA, 1977.
78. **Yashiro, R.,** The effect of *Artemia* fed different diets on the growth and survival of *Penaeus monodon* (Fabricius) postlarvae, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 447.
79. **San Feliu, J. M., Munor, F., Amat, F., Ramos, J., Pena, J., and Sanz, A.,** Cultivo experimental de larvas de crustaceos y peces en tanques, *Inf. Tech. Inst. Invest. Pesq.*, 36, 76, 1976.
80. **Rodriguez, A. M.,** Expérience de ponte et d'élevage de larves et de postlarves de crevettes *Penaeus kerathurus* (Forskal, 1779), *Etud. Rev.*, 55, 49, 1976.
81. **Guimaeres, J. I. and Lira do Rego, F.,** The use of freeze-dried *Artemia* as food source for penaeid shrimp larvae, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 479.
82. **Palmegiano, G. B. and Trotta, P.,** *Artemia salina* as pabulum for growing penaeids under laboratory conditions, *Aquacult. Eng.*, 2, 173, 1983.
83. **Trotta, P., Villani, P., Palmegiano, G. B., Forneris, G., and Sarra, C.,** Laboratory-grown *Artemia* as reference food for weaning fish fry and shrimp postlarvae, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 459.
84. **Flores, T. A.,** Preliminary production results of *Artemia* to be used in local shrimp farming in La Paz (Mexico), in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 283.
85. **Salgado, I.,** personal communication, 1988.
86. **Janata, W. R., Bell, D. J., and Keller, P. D.,** The DSIR-*Artemia* harvesting programme, in *Artemia Research and its Applications*, Vol. 3, Sorgeloos, P., Bengtson, D. A., Decleir, W., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1987, 221.
87. **Vanhaecke, P. and Sorgeloos, P.,** International Study on *Artemia*. XLVII. The effect of temperature on cyst hatching, larval survival and biomass production for different geographical strains of the brine shrimp *Artemia* spp., *Ann. R. Soc. Zool. Belg.*, 7, 119, 1989.
88. **Vanhaecke, P. and Sorgeloos, P.,** International study on *Artemia*. XIV. Growth and survival of *Artemia* larvae of different geographical origin in a standard culture test, *Mar. Ecol.*, 3, 303, 1980.
89. **Von Hentig, R.,** Einfluss von Salzgehalt und Temperatur auf Entwicklung, Wachstum, Fortpflanzung und Energiebilanz von *Artemia salina*, Ph.D. thesis, University of Hamburg, Germany, 1970, 136.
90. **Claus, C., Benijts, F., Vandeputte, G., and Gardner, W.,** The biochemical composition of the larvae of two strains of *Artemia salina* (L.) reared on two different algal foods, *J. Exp. Mar. Biol. Ecol.*, 36, 171, 1979.
91. **Schauer, P. S., Johns, D. M., Olney, C. E., and Simpson, K. L.,** International Study on *Artemia*. IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia*, in *The Brine Shrimp Artemia*, Vol. 3, Persoone, G., Sorgeloos, P., Roels, O., and Jaspers, E., Eds., Universa Press, Wetteren, Belgium, 1980, 365.
92. **Dobbeni, A.,** personal communication, 1983.